CHAPTER 19

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Renal Disease in Pregnancy

EFFECTS OF NORMAL PREGNANCY ON THE KIDNEY

Structural Changes in Pregnancy

Pregnancy normally induces changes in kidney function. In some women, complications of pregnancy may cause renal disease, and even normal pregnancy can exacerbate preexisting renal disease. Kidneys increase in volume, as much as 70% by ultrasound studies, during pregnancy, largely secondary to increased fluid content (1). The calyces and ureters dilate markedly during pregnancy, beginning as early as the 7th week and progressing gradually until term. By 1 week postpartum, these portions of the collecting system return to the prepregnant state in one third of women. In an additional one third, this dilation reverts by 1 month postpartum, and nearly all remaining patients return to normal by 2 months postpartum (2). The dilation is nearly always more prominent on the right, possibly because of the abrupt angle of the right ureter as it descends into the pelvic cavity. These changes may be influenced by several factors. The enlarged uterus physically contributes to ureteral compression. The upper ureter also develops increased tone during pregnancy because of hypertrophy of its smooth muscle and hyperplasia of surrounding connective tissue (3). These factors may also contribute to urinary tract infection (UTI) by increasing urinary volume within the collecting system and by increased stasis (see Urinary Tract Infections, p. 816).

Understandably, there are very limited renal biopsy data from any normal populations without renal disease, and in particular of normal pregnant women. No microscopic morphologic changes are usually observed in the kidney during normal pregnancy (1). *Endotheliosis*, a term used to describe the characteristic widespread endothelial cell swelling seen in preeclampsia/eclampsia (see Preeclampsia and Eclampsia, p. 819), may be present in very segmental areas even in normal pregnancy. In contrast, a small study in 1960 investigated five healthy pregnant controls, none of whom showed signs of endotheliosis (4).

Thus, the presence of endotheliosis may not be pathognomonic for preeclampsia; rather, its extent may be what distinguishes the pathologic condition (5).

Functional Changes in Pregnancy

Pregnancy is a volume-expanded condition, with increased circulating volume and interstitial volume and apparent resetting of volume-sensitive receptors to sense this expansion as normal. Thus, in normal pregnancy, blood pressure decreases despite increased volume as a result of decreased peripheral resistance, even though cardiac output increases by 30% to 50% by the end of the second trimester (1). Effective renal plasma flow increases 60% to 80% during pregnancy, with slightly less increase, by about 50%, in the glomerular filtration rate (GFR) (1). Direct micropuncture measurements in animals during pregnancy show moderate renal vasodilatation with similar reduction in resistances of afferent and efferent arterioles; thus, glomerular pressure is not increased. Direct measurements of glomerular pressures are not possible in humans. However, functional studies based on dextran sieving in normal and preeclamptic pregnancy have shown that increased filtration in late pregnancy was associated with increases in renal plasma flow and in the ultrafiltration coefficient, K_f, whereas in preeclamptic late pregnancy, there was a loss of permselectivity with accompanying decreases in K_f and renal plasma flow (6). Functional assessment with computation of K_f was also done in another study of patients immediately postpartum and the 2nd week after birth. Even at postpartum day 1, there was marked glomerular hyperfiltration, with data suggesting that decreased glomerular capillary oncotic pressure was the main determinant of this change. These changes resolved largely by postpartum week 2 with increases in oncotic pressure to supranormal levels, and thus, GFR was then only modestly elevated. Theoretical analysis suggested that transcapillary hydraulic pressure and/or increased K_f would have to be present to account for this persistent hyperfiltration (7). In an additional group of pregnant women studied in late pregnancy and 4 months postpartum, increased GFR was contributed to both by increased renal plasma flow and decreased glomerular oncotic pressure, with calculated increased K_f (8).

Possible hormonal mediators of the gestational increase in the GFR include primary gestational hormones (progesterone). However, progesterone, administered exogenously in pregnant animals, has no direct influence on renal hemodynamics. Other candidate vasoactive hormones that may change and affect renal vasodilatation include prostaglandins, the renin-angiotensin system, atrial natriuretic peptide, relaxin, and endothelin. Conclusive evidence is lacking to implicate specific mediators of altered renal hemodynamics in pregnancy.

Tubular function is also altered during pregnancy. Sodium retention occurs gradually over the course of pregnancy. Although the precise site of action has not been defined, this appears to be due largely to increased distal nephron reabsorption (1). The exact mechanisms underlying the increased retention of sodium have not been delineated, although effects of increased estrogen, placental lactogen, prolactin, growth hormone, desoxycorticosterone, renin-angiotensin, and aldosterone may contribute (1). Effects on potassium are dissociated from these effects on sodium, and tubular potassium loss is not excessive.

Proteinuria is increased in normal pregnancy, with increase in nondiscriminatory shunt pathways, that is, pathways that allow passage of charged, noncharged, and variably sized molecules, and an upward shift in pore size distribution deduced by dextran-sieving data (8). Proteinuria greater than 0.3 g/24 hours or urine protein:creatinine ratio $\geq$0.3 is regarded as abnormal during pregnancy by American Society of Hypertension guidelines (9).

Hematuria occurs frequently in normal pregnancy. In a case-control prospective study of 902 women, 20% had dipstick-positive hematuria on at least two occasions during pregnancy. There was no increased development of preeclampsia, gestational hypertension, or small-for-gestational-age baby in those with versus those without hematuria. Thus, transient hematuria during pregnancy seldom signifies a disorder with likely adverse effects on pregnancy (10).

Glycosuria also occurs commonly in pregnancy and returns to normal within 1 week postpartum (1). This finding appears related to change in tubular function and does not necessarily reflect glucose intolerance. The increased glucose in urine may facilitate development of bacteriuria (see section Incidence and Risk Factors for Bacteriuria). Tubular reabsorption of uric acid is decreased early in pregnancy and leads to relative hypouricemia at this stage. In contrast, preeclamptic women show hyperuricemia (see Preeclampsia and Eclampsia, p. 819). Thus, an increased serum uric acid level is a useful indicator of preeclampsia early in pregnancy. However, uric acid reabsorption increases normally in the third trimester, and serum levels then approach or exceed nonpregnant levels (1). Hypercalciuria also occurs commonly in pregnancy, yet urolithiasis is uncommon. This phenomenon may be due to the accompanying increase of nephrocalcin, a protein that can inhibit urinary crystallization. Additional factors that contribute to a decreased risk for calcium stone formation in pregnancy include increased citrate and magnesium excretion (1).

URINARY TRACT INFECTIONS

Incidence and Risk Factors for Bacteriuria

Significant numbers of bacteria can be found in urine cultures in patients without clear clinical manifestations of UTI. This situation occurs particularly in women and has been called asymptomatic, or covert, bacteriuria. Its importance lies in its relation to overt UTI and in particular to the associated risks in pregnancy for the mother and fetus. In pregnancy, the prevalence of asymptomatic bacteriuria ranges from 2% to 10% (11). This prevalence is comparable to the rate in sexually active nonpregnant women of reproductive age. Thus, pregnancy does not necessarily predispose to the development of asymptomatic bacteriuria, at least in developed countries (see later this section). Rather, it is possible that detection of this asymptomatic condition is increased during pregnancy.

The incidence of bacteriuria during pregnancy hits a peak between the 9th and 17th weeks of gestation. The dilation of the collecting system that begins as early as the 7th week (see p. 815) is thought to contribute to the development of upper UTI, because infection localizes to the dilated side in bacteriuric women with unilateral ureteral and calyceal dilation (2,11). Both increased urine volume within the dilated collecting system and increased urinary stasis (see p. 815) likely contribute to this increased risk of infection.

Increased frequency of asymptomatic bacteriuria is seen with a history of previous UTI, increased sexual activity, diabetes, and structural abnormalities of the urinary tract. Some,

but not all, studies suggest that age, parity, race, and sickle cell trait are also linked to increased asymptomatic bacteriuria (11). In contrast to a 6% prevalence of asymptomatic bacteriuria in pregnancy in otherwise healthy women, the rate was 12.2% in diabetic women and 18.7% in women with a history of previous UTI (11). Recent observational studies show constant and similar rates of asymptomatic bacteriuria in pregnancy in developing countries and in developed countries. The prevalence of asymptomatic bacteriuria in pregnancy is increased in patients with low socioeconomic status, but the mechanisms underlying these observations are unknown (11).

Gestational glycosuria and decreased potassium stores may facilitate bacterial growth in urine (2). Although not peculiar to pregnancy, the virulence factors of organisms and the status of the host's uroepithelium are clearly important. The formation of a biofilm and bacterial ability to adhere to and invade the urothelium are key for UTI occurrence. Virulent uropathogenic *Escherichia coli* (UPEC) typically adhere and activate host responses, including an innate immune response and Toll-like receptor signaling. Type 1 fimbriae bind to various epitopes, including Tamm-Horsfall glycoprotein mannosylated sites, secretory IgA, or uroplakins in the bladder urothelium. P fimbriae mediate mannose-resistant adherence of UPEC and are associated with acute pyelonephritis (12). When bacteria adhere via P fimbriae, the innate immune response is activated with increased Toll-like receptor 4 signaling and cytokine and neutrophil recruitment. Patients with decreased or defective Toll-like receptor 4 may thus have asymptomatic bacteriuria. Genetic variations in these response elements may thus favor asymptomatic bacteriuria versus acute pyelonephritis as outcomes of bacteria in the urine (13).

The most common organism causing bacteriuria during pregnancy is *E. coli*, followed by other gram-negative bacteria (*Klebsiella* sp, *Enterobacter* sp., *Proteus* sp.). Other reported organisms include *Staphylococcus saprophyticus*, which promotes stone formation as well. If fastidious organisms such as *Ureaplasma urealyticum* and *Gardnerella vaginalis* are also selected for by culture conditions, up to 25% of pregnant patients may show bacteriuria. However, these latter organisms have not been shown to play a pathogenic role (14).

Asymptomatic or Covert Bacteriuria

Effects on the Kidney

Other kidney abnormalities may develop or may be detected in patients with asymptomatic or covert bacteriuria during pregnancy (15–21). First is the issue of underlying structural urologic abnormalities or evidence of chronic pyelonephritis. Pregnant patients with asymptomatic bacteriuria showed a high prevalence of urinary tract abnormalities in a series of patients with urologic studies performed postpartum, also observed in a more recent study where only 14.3% of pregnant women with acute pyelonephritis showed normal kidneys by ultrasound (15). Ten percent of these patients showed evidence of chronic pyelonephritis (14). In several large series of pregnant women with asymptomatic bacteriuria who were followed for 2 to 14 years (14), bacteriuria was present in 16% to 29% during follow-up, and radiologic evidence of chronic pyelonephritis was seen in 9% to 29%. Many patients had underlying abnormalities, including bifid pelvis and ureteric duplication, and 27% showed signs of chronic pyelonephritis, such as calyceal blunting, diminished cortical thickness, and irregular renal contour. Despite these significant abnormalities, long-term follow-up of patients with asymptomatic bacteriuria during pregnancy has shown only rare cases of deterioration of renal function (20,21).

Assessment of these various series is difficult for several reasons. First is the danger of considering bacteriuria of pregnancy a specific complication acquired during pregnancy. The appreciable prevalence of asymptomatic bacteriuria in children and nulliparous women raises the possibility that asymptomatic bacteriuria may be present before conception. Bacteriuria was present in 8% of nulliparous married women compared with 6.6% of pregnant women (19). Many girls with asymptomatic bacteriuria have bacteriuria when they grow up and become pregnant. From 40% to 64% of women with a history of asymptomatic bacteriuria during childhood have bacteriuria during pregnancy (20,21). Further, it is difficult to assess whether asymptomatic bacteriuria is detrimental to kidney function because of the lack of prepregnancy measurements of renal function or serial radiographic studies. In addition, the radiologic abnormalities described in the previous paragraph in many patients with asymptomatic bacteriuria could indicate a predisposition to infection or a consequence of infection, or they may even be unrelated to infection. Whether the renal scars detected radiologically reflect injury from bacteriuria occurring in adulthood or from childhood UTI also is not clear.

Second is the potential of asymptomatic bacteriuria to cause acute pyelonephritis and its associated complications, including preterm labor, low birth weight, and growth retardation. The incidence of acute pyelonephritis in a recent large study of pregnant women was 0.07%, less than that observed before routine screening for asymptomatic bacteriuria and treatment were instituted (11). Bacteriuria in pregnancy leads to acute symptomatic infection in 20% to 40% of patients (14,15). Conversely, women without bacteriuria early in pregnancy rarely develop symptomatic urinary tract disease. Successful treatment of bacteriuria largely prevents the development of acute pyelonephritis. The consequences of symptomatic UTI are discussed below.

Effects on Pregnancy

The effect of asymptomatic bacteriuria on pregnancy outcome was previously controversial. Higher rates of prematurity and intrauterine growth retardation in patients with asymptomatic bacteriuria were observed in older studies but were initially not uniformly confirmed (15). Recent large studies demonstrate an effect of asymptomatic bacteriuria on pregnancy with increased preterm delivery, intrauterine growth retardation, and low birth weight (15).

Symptomatic Urinary Tract Infection and Pyelonephritis

Consequences and Complications

The exact extent of the urinary tract that is involved by infection cannot be readily assessed without invasive tests. Therefore, the term *symptomatic bacteriuria* is used by many authors to encompass both acute pyelonephritis and symptomatic infection of the lower urinary tract. Although risk factors for bacteriuria may overlap with those in asymptomatic infection, as discussed in Asymptomatic or Covert Bacteriuria, the consequences differ. Symptomatic UTI has well-defined serious consequences during pregnancy. Acute pyelonephritis in pregnancy is associated with bacteremia in 10% and endotoxic shock in up to 3% of patients (15).

The ability to predict which patients with asymptomatic bacteriuria will develop symptomatic infection is not established. This inability relates in part to a lack of understanding of the mechanisms that allow asymptomatic bacteriuria to become symptomatic, presumably reflecting upper UTI in most patients. A history of previous symptomatic UTI greatly increases the risk of progressing from asymptomatic to symptomatic infection during pregnancy (15). Underlying abnormalities, such as reflux, may predispose to progression from asymptomatic to symptomatic infections. Despite the dilation of the collecting system that normally occurs in pregnancy, reflux is uncommon, detected in only 0% to 3%, and likely does not play a significant role in the development of symptomatic infection in most patients. However, the vast majority of patients, over 85%, with pyelonephritis during pregnancy had abnormal renal ultrasound, including, for example, hydroureter, hydronephrosis, or renal calculi, with normal ultrasound in only 14.3% (15). Acute pyelonephritis complicated 6% of pregnancies in patients with reflux in one series (16). Successful ureteral reimplantation in women with reflux and past symptomatic UTI did not, however, abolish complications in subsequent pregnancies: 57% had UTI, and 17% had pyelonephritis during later pregnancies (17). These findings indicate that reflux cannot be regarded as the only mechanism for development of upper UTI in these patients. It is possible that the apparent greater ease of ascending infection in pregnancy is due to mechanical changes in pregnancy and altered innate immune responses.

The importance of preceding asymptomatic infection in the development of symptomatic infection has been clearly demonstrated. Screening programs to detect and treat asymptomatic bacteriuria in pregnancy decrease the incidence of pyelonephritis (15). This effect was shown dramatically by Kincaid-Smith and Bullen (22), who noted a 3.3% prevalence of symptomatic bacteriuria in a treated group of patients with asymptomatic bacteriuria versus a prevalence of 36.6% in a placebo-treated group.

The prevention of pyelonephritis during pregnancy has important implications for subsequent pregnancies as well. Patients treated for acute pyelonephritis have frequent occurrences of symptomatic UTI, both later in pregnancies and when not pregnant. This situation may result from increased susceptibility to repeated infection once parenchymal scarring has occurred. Radiologic findings of chronic pyelonephritis were present at follow-up in 14% to 27% of women who had bacteriuria detected during pregnancy (16,18). Women with renal scars resulting from childhood UTI had a higher incidence of bacteriuria during pregnancy than when scarring was absent (47% vs. 27%) (19). Patients with renal scars also had a significantly increased relative risk of hypertension, preeclampsia (up to 7.6-fold increase), and fetal morbidity during subsequent pregnancies compared with controls (20,21). In contrast, patients with vesicoureteral reflux but without scars had no increased risk of gestational hypertension or preeclampsia, although the risk of UTI was increased. This risk was not changed by ureter reimplantation (20). However, whether pregnancy affects the course of the underlying condition or merely increases the detection of infection and renal scarring has not been proven.

Symptomatic UTI has serious consequences for the pregnancy as well as for the mother. Prematurity occurred in 20% of pregnant women with acute pyelonephritis. There is controversy whether treated pyelonephritis also has adverse pregnancy effects (15). Older data showed that the fetal mortality rate was increased, but this was not confirmed in more recent studies (11). Production of phospholipase A_2 by the infecting organism may contribute to preterm labor by liberating arachidonic acid esters from phospholipids of infected amnionic and chorionic membranes, thus increasing levels of prostaglandins E_2 and F_2, which can trigger labor (11). A role for cytokines induced by infection, such as tumor necrosis factor and cachectin, has been suggested in preterm labor (23).

An additional serious complication of pyelonephritis in pregnancy is the occurrence of pulmonary insufficiency resembling adult respiratory distress syndrome, estimated to occur in about 7% of pregnant patients with acute pyelonephritis (11). This pulmonary injury may be related to endotoxin, prompted by lysis of bacteria in response to treatment.

Treatment

Cost-effectiveness and cost-to-benefit analyses show that screening for and treatment of asymptomatic bacteriuria prevents pyelonephritis in pregnancy and decreases preterm delivery (11). Currently, screening cultures are recommended for all pregnant women, preferably during the 16th gestational week for greatest potential impact on pregnancy outcome. Once asymptomatic bacteriuria is detected and treated, urine cultures should be repeated monthly throughout pregnancy, because as many as one third will have relapse or recurrence during pregnancy. If more than one relapse occurs, intravenous pyelography is recommended after 6 weeks postpartum. Urologic evaluation during pregnancy has been recommended if asymptomatic bacteriuria recurs or if appropriate treatment fails to eradicate bacteriuria. Either setting indicates a possible underlying urologic anomaly, obstruction, or abscess. Treatment of pyelonephritis is approached in a similar manner to that in nonpregnant patients.

HYPERTENSIVE DISORDERS OF PREGNANCY

Hypertension is a common problem during pregnancy, affecting 6% to 8% (24). Hypertension in pregnancy is defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg (24). It may be preexisting, or caused by, or exacerbated by the pregnancy. These complexities have led to difficulties in establishing the causes of hypertensive conditions in pregnancy. Although many classifications have been proposed, this discussion is based on the classification initially proposed by Lindheimer and Katz and slightly modified by the National High Blood Pressure Education Program Working Groups on High Blood Pressure in Pregnancy (as reviewed in reference (25). These disorders are divided into chronic hypertension, gestational hypertension, preeclampsia superimposed on chronic hypertension, and preeclampsia or eclampsia.

Chronic Hypertension

The most common cause of hypertension during pregnancy is preeclampsia (see p. 819), followed by essential hypertension and secondary causes, among which renal disorders are the most common. The prevalence of chronic hypertension in pregnancy is about 3% in the United States (25,26). In patients who develop hypertension, but not preeclampsia during pregnancy, underlying renal disease should be considered (see below).

Not surprisingly, populations with a higher general incidence of hypertension also show a higher incidence of hypertension detected during pregnancy. Thus, in a study from South Africa, black women showed a higher prevalence of hypertension during pregnancy than that seen in other predominantly white populations: 23% versus 7% to 10% (27).

Although pregnant women with mild essential hypertension do have a greater risk of developing superimposed preeclampsia, most of these patients do not experience complications during pregnancy. Treatment of hypertension has not been shown to decrease risk for preeclampsia (25). If hypertension in pregnancy is associated with proteinuria greater than 500 mg/d, even in the absence of overt preeclampsia, increased maternal complications and worse fetal outcome are seen (28).

Poor outcomes and even death may occur when hypertension is secondary to scleroderma, cocaine ingestion, or pheochromocytoma. Fibromuscular dysplasia is a frequent cause of renal artery stenosis in young women, and it should be considered when hypertension precedes pregnancy. An apparent paradox is observed in patients with renal artery stenosis: they may actually have reduced hypertension during pregnancy because altered tubular function in pregnancy leads to less potassium loss and less induction of aldosterone (29). Correction of the stenosis by angioplasty with resolution of hypertension decreases risk of fetal and maternal complications (30).

In patients with chronic hypertension without superimposed preeclampsia, most (more than 85%) experience uncomplicated pregnancies. However, birth weights are lower and the perinatal mortality rate is increased in patients with hypertension due to underlying renal disease or other secondary cause, older than 40 years, diabetic, with previous pregnancy loss, or history of stroke compared with normotensive patients (25). Whether treatment of mild chronic hypertension affects the risks of premature delivery and superimposed preeclampsia is controversial. Methyldopa has been recommended as the drug of choice. The angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, which are widely used in many other settings of hypertension and cardiovascular disease, are contraindicated in pregnancy because of their association with both neonatal acute renal failure and birth defects (26).

Preeclampsia Superimposed on Chronic Hypertension

Patients with this disorder have underlying hypertension in combination with a further elevation of blood pressure and the appearance of or increase in proteinuria, that is, preeclampsia. Edema may also develop. In a series of 13 women with preexisting essential hypertension or renal disease who were suspected of having superimposed preeclampsia clinically, only 7 of the biopsies showed typical changes of preeclampsia (31). About 25% of pregnant women with chronic hypertension develop superimposed preeclampsia (25). Preeclampsia in this population frequently occurs in midpregnancy or early in the third trimester. An acute medical emergency can arise in this setting, and fetal outcome may be jeopardized (25).

Gestational Hypertension

Gestational hypertension is defined as hypertension after 20 weeks estimated gestation in women without previous hypertension and without proteinuria (25). In various studies, 15% to 45% of these patients developed preeclampsia (25). Blood pressure normalizes within 10 days after delivery, but late hypertension may recur in subsequent pregnancies (29).

Preeclampsia and Eclampsia

Clinical Findings

Toxemia of pregnancy, including both preeclampsia and eclampsia, occurs in up to 8% of pregnancies, with an increasing incidence, and especially higher incidence in developing countries (32,33). True eclampsia, defined as the occurrence of convulsions in association with the signs and symptoms of preeclampsia, was found in nearly 4.9 per 10,000 pregnancies in one study from the United Kingdom (34). In lower-income settings, 2.3% of women with preeclampsia developed eclampsia, compared to only 0.8% of women in countries with higher income (35). The disease is named eclampsia (from the Greek eklampsis, meaning sudden flashing, i.e., lightening) because of the sudden occurrence of onset of convulsions in association with the signs and symptoms of preeclampsia. Preeclampsia is primarily a disease of the nullipara and manifests usually after the 20th week of the first gestation. Before the 20th week of gestation, preeclampsia is most frequently associated with molar pregnancy or its degeneration. When the disease occurs for the first time in multiparas, it is typically associated with multiple-birth gestation, fetal hydrops, preexisting vascular disease, or renal disease (36). A large study systematically reviewing controlled studies published from 1966 to 2002 examined unadjusted relative risk for development of preeclampsia based on factors that could be determined at the initial visit. Increased risk of preeclampsia was seen in patients with a history of previous eclampsia, antiphospholipid antibodies, preexisting diabetes, multiple pregnancy, nulliparity, family history of preeclampsia, increased diastolic blood pressure at initial visit, increased body mass index before pregnancy or even at initial visit, and maternal age of 40 years or older. There was also an increased risk with an interval of 10 years or more since a previous pregnancy, with autoimmune disease, renal disease, and chronic hypertension (37).

Preeclampsia is characterized by hypertension (greater than 140/90 mm Hg or marked increase over baseline), proteinuria, and edema, especially of face and hands. The labile hypertension and altered circadian rhythm of blood pressure in preeclampsia, with higher levels at night, may lead to difficulty in detecting the increased blood pressure if blood pressure is measured only during the day (1). Wide fluctuations in blood pressure, even including the normal range, are thought to reflect increased sensitivity to vasoconstrictors (7). Proteinuria is usually not marked, but the nephrotic syndrome with protein loss of up to 23 g/d has been reported (38). The condition occasionally progresses to a convulsive phase, termed *eclampsia*, which may be life threatening. Eclampsia occurs in about 5 of 10,000 live births. The incidence has declined, perhaps due to urgent delivery in the preeclamptic patient or use of magnesium sulfate (39). Preeclampsia and eclampsia follow only hemorrhage as a cause of pregnancy-associated death in women worldwide (35). Data from the United Kingdom showed that nearly 2% of eclamptic women died, as did 7% of their offspring (34). Eclampsia may also develop without an obvious preceding stage of preeclampsia (34). In some patients, hypertension, proteinuria, and convulsions may occur in the immediate postpartum period (so-called "late postpartum eclampsia"). Preeclampsia and eclampsia may occur de novo,

or they may be superimposed on preexisting hypertensive disorders, as mentioned below.

In preeclampsia, the GFR is decreased, but this change may not be detectable because of the preceding normal increase of GFR seen with pregnancy. Detailed studies have now elucidated further the functional implications of the endotheliosis lesion that characteristically occurs in preeclampsia/eclampsia. Patients with preeclampsia had markedly decreased GFR levels, 91 ± 23 versus 149 ± 34 mL/min/1.73 m^2 in normals, whereas renal plasma flow and oncotic pressures were similar to that in normal pregnancy. Combined physiologic and morphometric studies were used to estimate the glomerular ultrafiltration coefficient (K_f) in normal pregnancy and preeclampsia. The decrease of both density and size of endothelial fenestrae and the substantial subendothelial accumulation of lucent material were postulated to lower glomerular hydraulic permeability in preeclampsia. The presence of cellular interposition, that is, infiltrating cells, usually monocyte/macrophages, interposed between the endothelium and the GBM, also was postulated to contribute to decrease in effective filtration surface area, so that single nephron K_f estimated in this matter was well below control. These changes occurred despite significantly larger glomerular volume in preeclampsia so that actual filtration surface area was reduced to only a minor extent (40). These morphologic findings suggest that structural lesions could be a major contribution to decreased GFR owing to the decrease in K_f and that hemodynamic changes may have less influence than previously thought (33).

Hyperuricemia resulting from decreased clearance predates heavy proteinuria in nearly all cases of preeclampsia. Sodium is retained, contributing to edema. Peripheral resistance and vascular sensitivity to angiotensin II are increased (41). This finding is in contrast to the blunted responsiveness to angiotensin in normal pregnancy with up-regulation of all renin-angiotensin components. The Ang (1–7) metabolite of angiotensin II counteracts many of angiotensin II's actions and is increased in normal pregnancy, in contrast to a decrease in preeclampsia (41). Red blood cell fragmentation may occur even in the absence of the postpartum hemolytic-uremic syndrome (see Chapter 18). Platelet counts are decreased, whereas fibrin degradation products and fibronectin levels in plasma are increased, indicative of fibrinolysis and vascular injury (42). Despite the vascular injury, serum complement levels are usually not different in normal and preeclamptic pregnancies (43). Urinalysis is nonspecific and may show occasional red blood cells, white blood cells, and casts.

Differential Diagnosis

Because the clinical findings of preeclampsia are largely nonspecific, misclassification occurs commonly. Thus, several studies of postpartum renal biopsies in women thought to have preeclampsia clinically showed other renal lesions in as many as half these patients (38,40,44,45). Underlying renal biopsy lesions included chronic glomerulonephritis, tubulointerstitial lesions, membranous glomerulopathy, sickle cell nephropathy, acute poststreptococcal glomerulonephritis, minimal change nephrotic syndrome, and diabetic nephropathy, in descending order of frequency. In some patients, lesions of preeclampsia were superimposed on specific findings of these renal diseases (31,46). The clinical diagnosis is inaccurate even in a large proportion of primiparas: 25% in one series of patients with clinically diagnosed preeclampsia actually had chronic glomerulonephritis (47). In patients with apparent preeclampsia before the third trimester, an especially high prevalence of underlying disease has been found. When preeclampsia was diagnosed clinically before 37 weeks' gestation, 67% of patients in one study had disease other than preeclampsia, including glomerulonephritis (IgA nephropathy, other mesangial glomerulonephritis, reflux nephropathy, polycystic kidneys, and diabetes) and essential hypertension (48).

The effect of pregnancy on preexisting renal disease is discussed below on page 837. These patients represented a population referred for evaluation of possible renal disease postpartum, and many had hematuria. In one study, women with clinical diagnoses of either preeclampsia or gestational hypertension without clinical evidence of underlying disease during pregnancy were evaluated postpartum for evidence of renal disease. This clinical evaluation showed evidence of underlying renal disease in only 7 of the 87 (8%) women with a diagnosis of preeclampsia and in 16 of the 99 (16%) patients with apparent gestational hypertension (49). However, renal biopsy was performed only in the single patient who had hematuria in this series (demonstrating thin basement membrane lesion). Two points emerge from these studies. First, the incidence of underlying renal disease varies among patients with apparent preeclampsia, depending on patient referral and selection criteria. Second, the exact structural lesions underlying renal dysfunction in pregnancy cannot be known precisely in the absence of renal biopsy.

Nonrenal disorders may also be associated with preeclampsia-like syndromes. A case report describes the occurrence of a reversible preeclampsia-like syndrome during pregnancy in a hypothyroid patient (50). Renal biopsy showed enlarged, bloodless glomerular tufts with endothelial cell swelling and mesangial interposition, similar to findings seen in hypothyroidism without pregnancy and to the typical lesions of preeclampsia (see Pathologic Changes, pp. 821–825).

Course and Prognosis

Clinical signs and symptoms of preeclampsia and eclampsia resolve with cessation of pregnancy, typically within 24 hours of delivery. If the patient is near term, preeclampsia is best treated by induction of labor. If the fetus is immature, treatment with bed rest and sedation may allow continuation of the gestation unless findings of HELLP (*h*emolysis, *e*levated *l*iver enzymes, *l*ow *p*latelet count) syndrome (see HELLP syndrome, p. 832) are present. Intravenous magnesium sulfate is used for impending eclampsia because of its antihypertensive effects and amelioration of central nervous system symptoms of preeclampsia and eclampsia (1). Other treatment strategies focus on antihypertensives and fluid and electrolyte management. However, the basic nature of volume homeostasis in preeclampsia has not been defined. Some investigators view this as a condition with volume overload, whereas others point to evidence of decreased plasma volume in preeclampsia. Thus, diuretics and volume expansion have variously been advocated in the treatment of preeclampsia.

Hypocalcemia is observed in preeclampsia, and treatment of preeclamptic patients with calcium contributes to normalization of blood pressure. Prophylactic calcium supplementation also reduced the risk of gestational hypertension and preeclampsia, especially in developing countries, or in developing countries in women with low basal calcium

levels (51). Increased emphasis on the possible participation of thromboxane and prostacyclin in the pathogenesis of vasospasm and thrombocytopenia has led to several trials of low-dose aspirin for the prevention of preeclampsia. However, a specific, effective prophylactic approach has not been defined.

The consequences of preeclampsia and eclampsia are difficult to assess because of several factors. First, clinical diagnosis of preeclampsia is not accurate, as discussed in Differential Diagnosis (p. 820). Therefore, the long-term consequences of preeclampsia may reflect the course of underlying disease and not the pregnancy-induced injury. Second, clinical parameters are not sensitive indicators of renal injury, and long-term follow-up is necessary to realize fully the impact of an injury on progressive renal dysfunction. Finally, limited renal biopsy studies have been performed. Although definitive diagnosis of preeclampsia can be achieved only with renal biopsy, most clinicians do not advocate this procedure in this setting. To complicate matters further, the timing of biopsies in relation to the preeclampsia-associated injury varies greatly. Furthermore, even normal pregnancies may be associated with mild focal endotheliosis lesions in biopsies (5). Even heavy proteinuria typically resolves by 3 months postpartum, and the typical glomerular lesion of preeclampsia, that is, endothelial swelling, also appears to be completely reversible (see Pathologic Changes, below). In contrast, persistent proteinuria, hypertension, and abnormal urinalysis findings beyond 3 months postpartum suggest other underlying renal disease (see Differential Diagnosis) (24). Long-term, preeclampsia is linked to increased cardiovascular disease and increased chronic kidney disease (39). When preeclampsia only occurred in the first pregnancy, the relative risk for end-stage renal disease was 4.7, with further increase if preeclampsia developed in additional pregnancies (52). Severe preeclampsia is also associated with doubling of the risk of development of venous thrombosis compared to normotensive pregnancies. The risk of development of type 2 diabetes mellitus is also increased in women with a history of preeclampsia, even after controlling for other risk factors for development of diabetes (39).

Renal biopsy findings correlated with some of these late complications. When nephrosclerosis, that is, sclerosis of vessels and glomeruli, was present in the renal biopsy in preeclamptic patients, 74% of patients had hypertension on follow-up (53). In contrast, only 9.4% of preeclamptic patients without vascular lesions developed hypertension, not significantly different from age-, sex-, and race-matched control populations. These data were taken to suggest that persistent hypertension after preeclampsia reflected preexisting disease and was not a consequence of preeclampsia. However, because baseline biopsies are typically not available, this issue has not been directly proven. Limited studies with follow-up biopsies have directly examined the reversibility of renal lesions in preeclamptic patients. In a small study of patients with nephrosclerosis detected in immediate postpartum biopsies, follow-up biopsies revealed persistent arteriolar changes, and four of these patients developed hypertension again in subsequent pregnancies (54). Taken together, these data suggest that arteriolar sclerosis may be irreversible and associated with increased risk for subsequent hypertension. Furthermore, based on the nature of the morphologic changes, the presence of arteriosclerosis or arteriolosclerosis in patients with preeclampsia likely reflects incipient hypertension-attributed nephrosclerosis.

Pathologic Changes

Historical Perspective

The glomerular changes of preeclampsia in autopsy specimens were initially described in detail by Löhlein in 1918 (55) and a few years later by Fahr (56,57). These investigators noted glomerular tuft swelling and expansion of the glomerular capillary wall, resulting in a bloodless appearance of the capillaries and capillary lumen occlusion. Subsequently, Bell (58) suggested that basement membrane thickening was responsible for the capillary occlusion. Sheehan (59) reported an extensive autopsy experience of patients with toxemia who died of apparent incidental obstetric complications. Remarkably, most of these autopsies were performed within 15 minutes to 2 hours after death, thereby avoiding artifacts from extensive autolysis (59,60). Sheehan, with only light microscopic studies available, noted glomerular endothelial cell swelling and fibrils between the endothelial cells and the basement membranes and postulated that endothelial cell changes accounted for the thickened capillary wall. Dieckmann et al. (61) were among the first to examine renal biopsy material in preeclampsia, and they confirmed many autopsy findings. These early light microscopic studies focused on the thickened glomerular capillary wall, thought to represent a thickened glomerular basement membrane. However, not until electron microscopic examination became available were these light microscopic observations further elucidated. Spargo et al. (62) and Farquhar (63) demonstrated by electron microscopy that the thickening of the glomerular capillary wall seen by light microscopy was not due to the glomerular basement membrane thickening in that the lamina densa of the glomerular basement membrane consistently was normal. However, these ultrastructural studies confirmed the presence of endothelial cell swelling. The term *glomerular endotheliosis* was coined by Spargo et al. for the described lesion (62). Other investigators confirmed the presence of glomerular endothelial cell swelling (64–67) and also described swelling of the podocytes (66,67). The occurrence of substantial glomerular subendothelial deposits visualized by electron microscopy was noted by Hopper et al. in 1961 (68). These deposits and the presence of a translucent subendothelial zone, possibly relating to fibrin deposition, were described in detail by Kincaid-Smith in 1973 (69). Furthermore, cellular interposition, that is, interposed portions of cells, most often monocyte/macrophages, between the endothelium and the GBM, was recognized to contribute to glomerular capillary wall thickening by Altchek (64) and Ishikawa (70).

Gross Appearance

Judging by the older reports on autopsies in cases of eclampsia, the kidneys show no distinctive changes visible to the naked eye (71). They are of normal size or are slightly enlarged; the cortex is pale and widened in the larger kidneys, whereas the glomeruli can often be seen to be unduly prominent and gray if looked at with a hand lens.

Microscopic Findings

The renal lesions are not substantially different in preeclampsia and eclampsia. The severity of morphologic alterations, which primarily affect the glomeruli, parallels the severity of clinical disease.

Glomeruli The glomeruli are diffusely slightly enlarged and swollen, and they appear bloodless (Fig. 19.1). In one postmortem study (71), glomerular size was approximately 10% larger

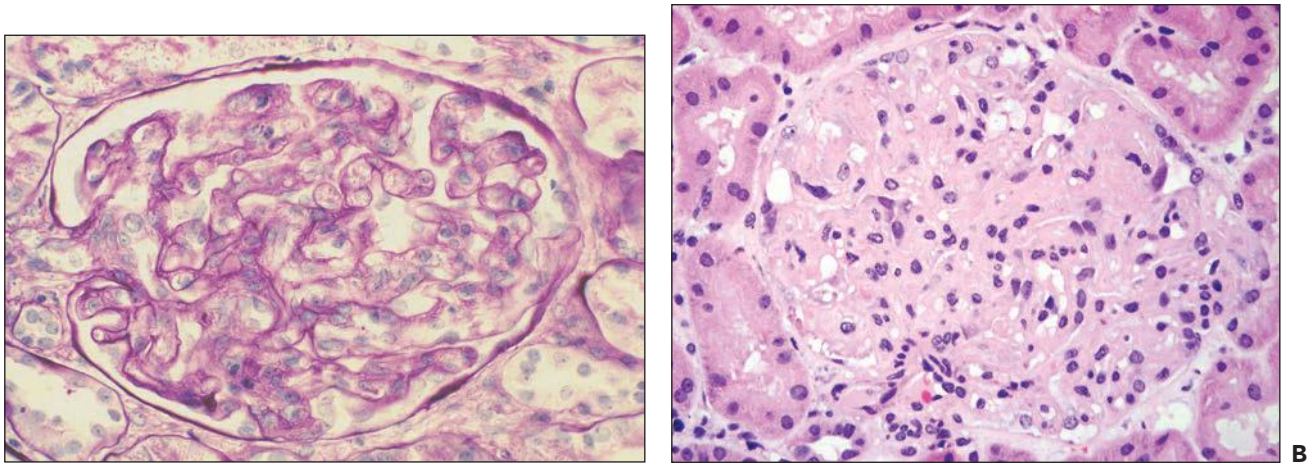


FIGURE 19.1 Glomeruli from patients with preeclampsia. A: The tuft appears bloodless, and the capillary lumina are reduced (so-called endotheliosis lesion). There is no cellular increase. (PAS, ×400.) **B:** In this patient, the tuft is bloodless and appears solidified (so-called endotheliosis lesion). Features are similar to those in (**A**), but the lesion is more severe. (H&E, ×450.) (Both courtesy of J. Charles Jennette, MD.)

than normal. The glomeruli show a characteristic lobular pattern from capillary expansion producing cigar-shaped lobules. The glomerular capillary lumina are narrowed or even obstructed because of marked mesangial and endothelial cell swelling and hypertrophy, so-called glomerular capillary endotheliosis (62). The glomerular capillary loops are dilated, so-called ballooning, particularly at the tubular pole. This may result in *herniation* of the glomerular tuft into the proximal tubule (so-called pouting; Fig. 19.2) (71). This extension involved about half of the glomeruli in this study (71), with capillary loops extending on average 50 μm into the proximal tubule. Glomerular capillary endotheliosis has been viewed by Gaber et al. (72) as pathognomonic for preeclampsia. Although it is well accepted that this lesion is part of the glomerular structural changes in preeclampsia, and the fully developed lesion as assessed by light and electron microscopy is characteristic, other lesions also occur in varying proportion, depending on the timing of the biopsy and the severity of the disease. Finally, endotheliosis-like lesions may not be uniquely present in preeclampsia. Normotensive patients with abruptio placentae showed lesions similar to, albeit milder than, those described in this section (73). Furthermore, in a study of renal biopsies performed 8 to 10 days postpartum in 32 women with gestational hypertension without proteinuria or clinical evidence of preeclampsia, 12 biopsies revealed the "specific" pattern of preeclampsia (74). A recent small study revealed that even some normal pregnancies may also be associated with small areas of endotheliosis-type lesions (5).

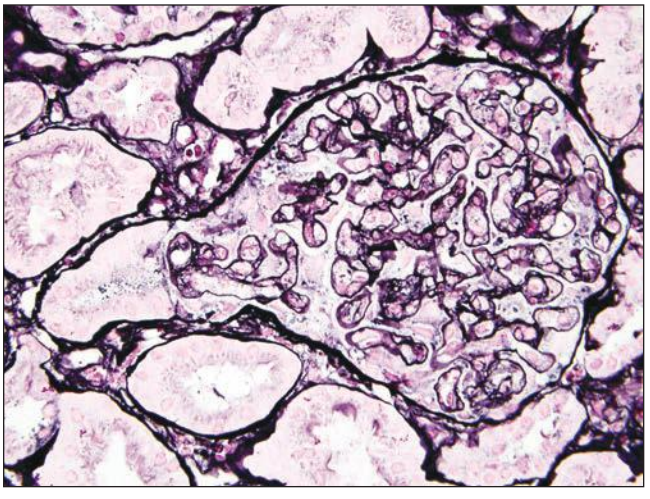


FIGURE 19.2 Glomerulus from a patient with preeclampsia showing similar features as in Figures 19.1, as well as herniation of the tip of the swollen glomerulus into the proximal tubule (so-called pouting), which is a characteristic but not specific feature of preeclampsia/eclampsia. (Jones silver stain, ×400.) (Courtesy of J. Charles Jennette, MD.)

Glomerular cellularity may be normal or slightly increased with only rare, or no, neutrophils. Mesangial and endothelial cell vacuolization with accumulation of fluid and lipid is visualized well on osmium-fixed toluidine blue–stained thick sections (45). The foamy, bubbly appearance of the glomerular endothelial, or to some extent mesangial, cells, or even cholesterol clefts at later stages, may relate to severe proteinuria (Fig. 19.3) (62). Fibrils within swollen glomerular endothelial cell cytoplasm adjacent to the basement membrane may be visualized (59,60). Mesangial cells and matrix may be mildly increased, and cellular processes may extend between the glomerular basement membrane and endothelium (cellular interposition). Cellular interposition is especially prominent in more severe disease and in the healing stage (69,71,75). Podocytes are swollen and prominent and may contain hyaline droplets that are positive for periodic acid-Schiff stain (Fig. 19.4). Crescents are seen only in the most severe cases of preeclampsia and eclampsia (60,71).

The glomerular capillary wall is thickened. Its components may be distinguished by the Alcian blue/periodic acid-Schiff reaction, which stains the glomerular basement membrane magenta and the cytoplasm blue. Silver stain may show a double contour, in part because of the interposition of cell cytoplasm processes (Fig. 19.5) (69). Glomerular basement membrane remodeling is associated with increased staining for

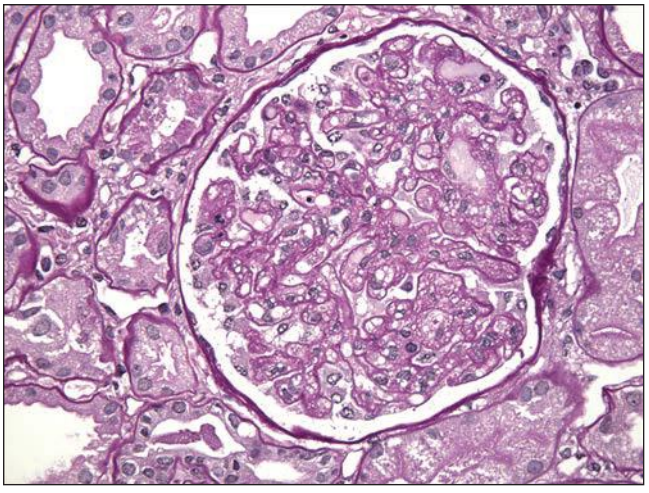


FIGURE 19.3 Glomerulus from a patient with preeclampsia revealing a pronounced bubbly appearance in the consolidated areas, caused by swollen endothelial cells and podocytes. (PAS, ×400.) (Courtesy of Vivette D'Agati, MD.)

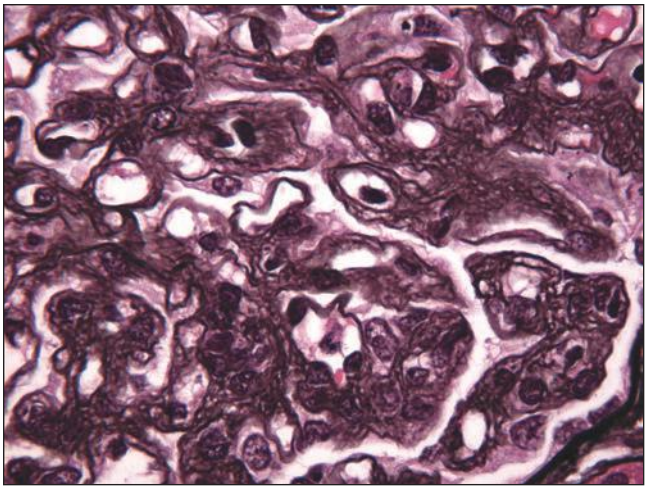


FIGURE 19.5 Glomerulus from a patient with preeclampsia showing widespread glomerular basement membrane double contours, revealed by electron microscopy (see Fig. 19.14) to be due to interposition and increased lamina rara interna. (Jones silver stain, ×1000.) (Courtesy of Vivette D'Agati, MD.)

laminin, type IV collagen, fibronectin, and proteoglycan (76). Carefully performed studies from biopsy material have revealed no distinct changes of the juxtaglomerular apparatus (77).

Time Course of Glomerular Lesions Endotheliosis, as described and coined by Spargo et al. (72), has often been regarded as the pathognomonic feature of preeclampsia. However, depending on the timing of the biopsy, other features may also be prominent. Various series have shown remarkable differences in frequency of detection of glomerular subendothelial deposits of fibrin or fibrinoid material (62,66,68,69,73,75). The analyses of Kincaid-Smith (75) of the evolution of lesions during pregnancy and postpartum indicate that these fibrin-like deposits may disappear quickly postpartum. Subendothelial deposits are most consistently present in biopsies done during the first few postpartum days (75). Rapid fibrinolysis can occur, and thus, fibrin would be less likely to be present after delivery and resolution of the preeclamptic injury cascade. Focal thrombotic microangiopathy may also be present in clinically severe cases of preeclampsia or full-blown eclampsia, mirroring the severity of the disease (Fig. 19.6).

Similarly, the extent of foam cells is correlated with the timing of biopsy. In postpartum biopsies, glomerular endothelial and, to a lesser extent, mesangial foam cells are nearly universally present as part of the endotheliosis lesion, a finding confirmed when biopsies were carried out within 12 days postpartum (62,78). In contrast, the biopsy study conducted over a longer time course by Kincaid-Smith and Fairley (79) and the extensive autopsy experience of Sheehan (59) documented only sparse foam cells. After the immediate postpartum period, cellular edema was reduced, whereas endothelial proliferation and increased mesangial cells persisted (80). Basement membrane double contours also appear to resolve rapidly after pregnancy, although these changes may persist for months in some cases (69,75). Focal segmental glomerulosclerosis occurs in some cases and is discussed on page 825.

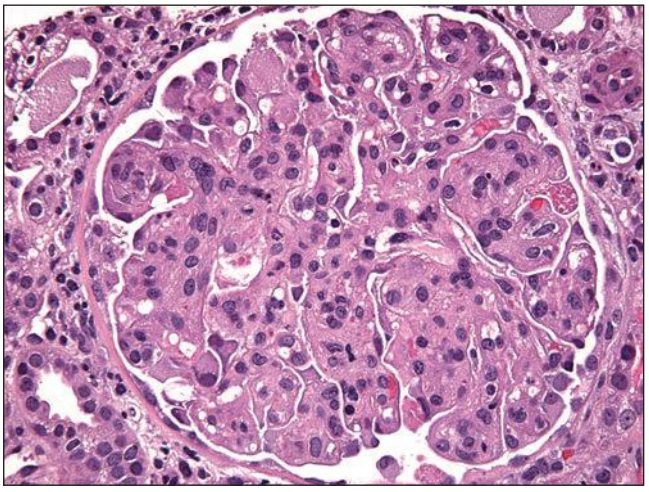


FIGURE 19.4 Glomerulus from a patient with preeclampsia revealing similar lesions as in Figure 19.3 and segmental hyaline droplets in the podocytes. (H&E, ×400.) (Courtesy of Vivette D'Agati, MD.)

Tubulointerstitium Tubulointerstitial changes are nonspecific. Atrophy of tubules and interstitial fibrosis parallel glomerular sclerotic changes. Proximal tubules may show protein reabsorption droplets and lipid droplets (45). Casts are present, particularly in collecting ducts, and some contain hemoglobin and stain for iron.

Blood Vessels Renal biopsies contain limited samples of large vessels, and the vascular changes observed in biopsies from preeclamptic patients are usually nonspecific. Multiple mechanisms may contribute to the lesions. Medial hypertrophy of renal and extrarenal interlobular arteries and arterioles in preeclampsia (44) may result from increased sensitivity to vasoactive substances such as angiotensin II (41) (see Clinical Findings, p. 819), which can exert trophic actions on vascular smooth muscle cells. The insudative lesions of hyalinosis of vessels

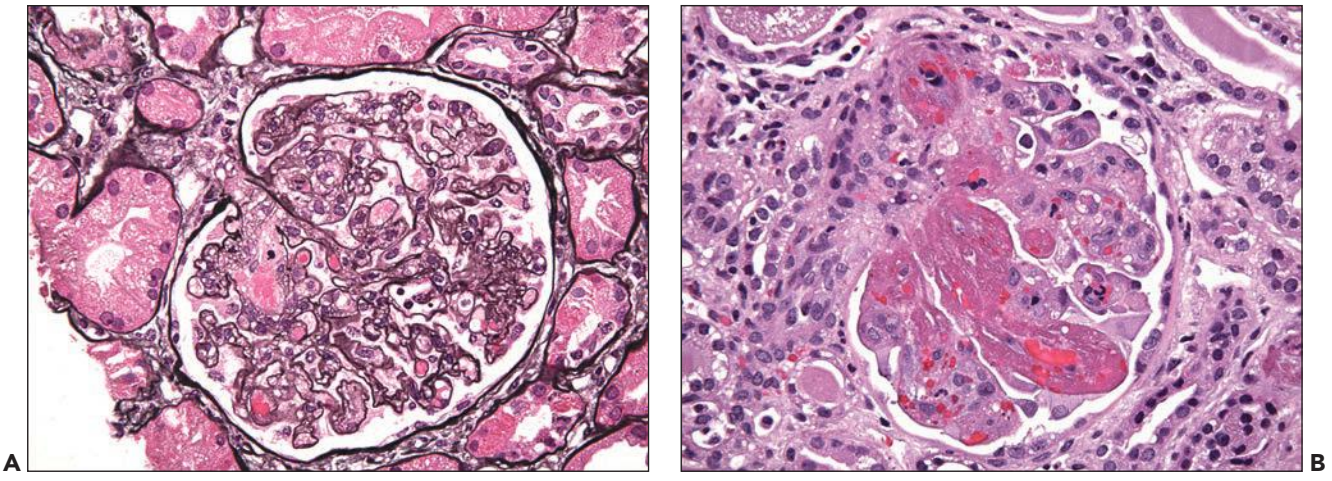


FIGURE 19.6 Glomeruli from patients with severe preeclampsia **(A)** and severe preeclampsia/eclampsia **(B)** revealing endotheliosis lesion and segmental early thrombotic microangiopathy. (**A,** Jones silver stain, ×400; **B,** H&E, ×400.) (Both courtesy of Vivette D'Agati, MD.)

possibly are related to endothelial injury and acute changes of blood pressure in preeclampsia. Findings of arteriosclerosis and arteriolosclerosis, with intimal fibrosis and thickening and reduplication of the elastic lamina, indicate the likelihood of preexisting nephrosclerosis (see p. 821) (53,72). This lesion of intimal sclerosis of arteries or arterioles was found in approximately one third of patients with pregnancy-related hypertension and in 10 of 14 patients with known hypertension or renal disease before pregnancy (44). Vascular lesions of malignant hypertension were found in some patients with preexisting renal disease and severe toxemia resulting in death, although hypertension was not at malignant levels clinically (81). These data could be taken to indicate that more severe toxemia occurs with more severe preexisting vascular lesions. On the other hand, severe vascular lesions of apparent "malignant hypertension" in these early reports may have been related to thrombotic microangiopathy (Fig. 19.7) (79). Severe vascular lesions, including possible vasculitis and thrombosis, can also occur when antiphospholipid antibodies are present (see p. 840).

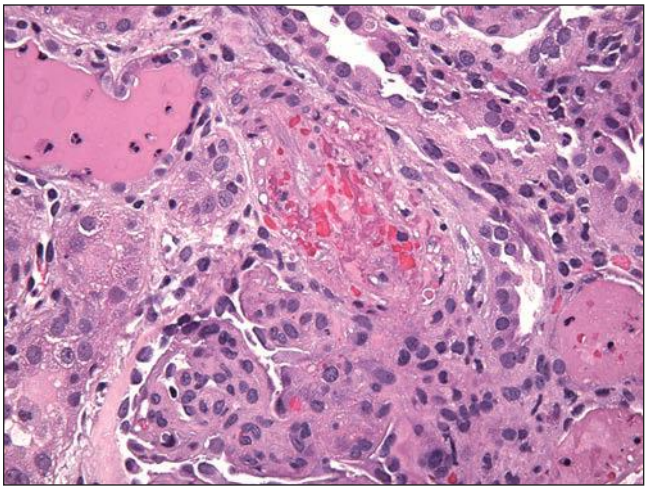


FIGURE 19.7 Patient with severe preeclampsia and glomerular endotheliosis lesion with severe arteriolar lesion with fibrinoid necrosis and intraluminal fibrin. (H&E, ×400.) (Courtesy of Vivette D'Agati, MD.)

Electron Microscopic Findings

Early biopsy studies with electron microscopy shed light on the precise cellular abnormalities in preeclampsia (62,63,66,82,83). Swelling of glomerular endothelial cells and, to a lesser extent, of mesangial cells is a prominent feature (Figs. 19.8 and 19.9). Lysosomes may be present in both cell types. Mesangial cells and matrix are increased, and mesangial cell interposition contributes to glomerular capillary wall thickening. Vacuolization, droplets, cytoplasmic strands, lipid, dense bodies, myelin figures, and increased numbers of cell organelles can be seen in both glomerular endothelial and mesangial cells (Fig. 19.10). Glomerular epithelial cell vacuolization and swelling are also frequent (66,67,79). Foot process effacement is seen only segmentally, and it does not appear to correlate with the degree of proteinuria (38). Epithelial cell droplets were shown by immunoelectron microscopy to contain immunoglobulin complement, fibrinogen, and larger amounts of albumin (75). Lipid droplets, both extracellular and intracellular, can be present.

Glomerular subendothelial and occasional mesangial vague densities are present, depending on the timing of biopsies (Figs. 19.11 and 19.12). The material can appear fibrillar, as more or less localized dense deposits, or as granular deposits. In severe cases, fibrin tactoids may be present in the glomerular subendothelial areas, the mesangium, and, rarely, the urinary space (Fig. 19.13) (44,45). Most investigators have identified fibrillar fibrin within dense glomerular subendothelial deposits (44,45,75,78,84). In one study, these deposits decreased in size postpartum and disappeared over the first 8 days after delivery (85). Fibrin- or fibrinogen-related breakdown products, fibronectin, and matrix components localized to these areas immunohistochemically (76,84). By immunoelectron microscopy, IgM staining also localized to areas of glomerular subendothelial electron-dense deposits in thickened loops (75).

The lamina densa is not increased in thickness. The glomerular basement membrane shows increased lucency of the lamina rara interna. Thus, by electron microscopy, several

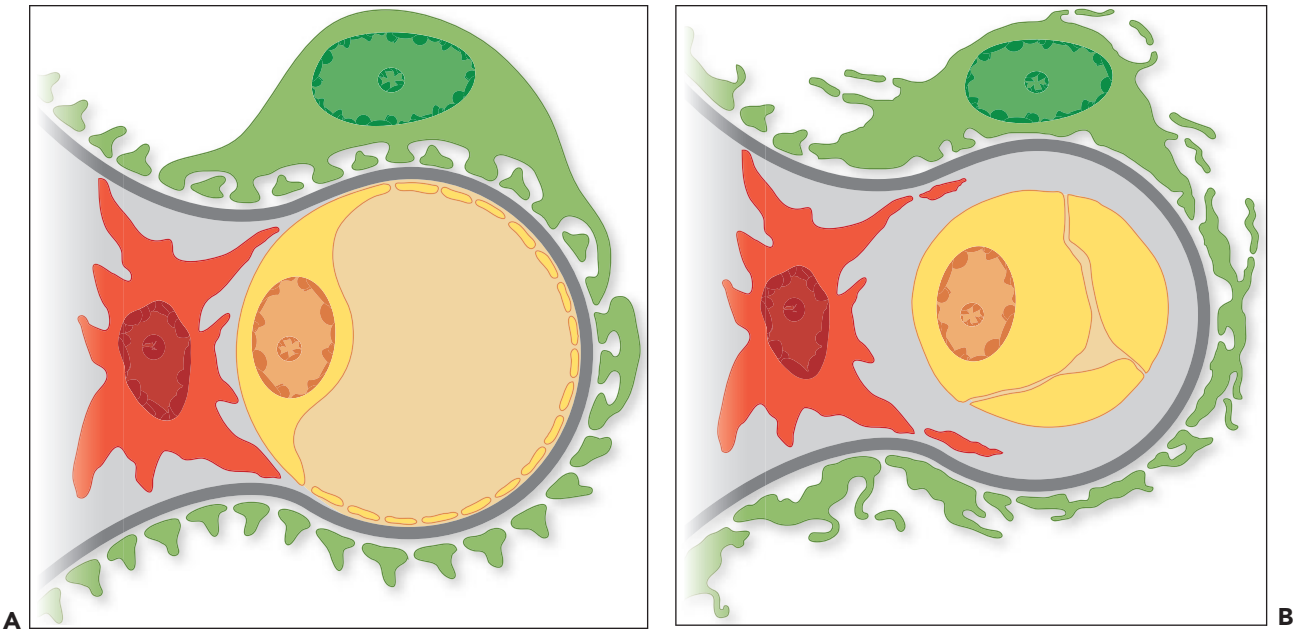


FIGURE 19.8 **A:** Diagram depicting one normal glomerular capillary. (Green = podocyte, dark gray = GBM, yellow = endothelial cell, red = mesangial cell, light gray = mesangial matrix). **B:** Diagram depicting a glomerular capillary in eclampsia with extensive endothelial swelling and expansion of the zone between the inner surface of the GBM and the endothelial cell.

elements are seen to contribute to the thickened glomerular capillary wall and reduplication of the basement membrane seen by light microscopy: endothelial cell swelling, mesangial cell interposition with new basement membrane formation (Fig. 19.14A), and an increased lucent zone of the lamina rara interna (Fig. 19.14B).

Immunofluorescence Findings

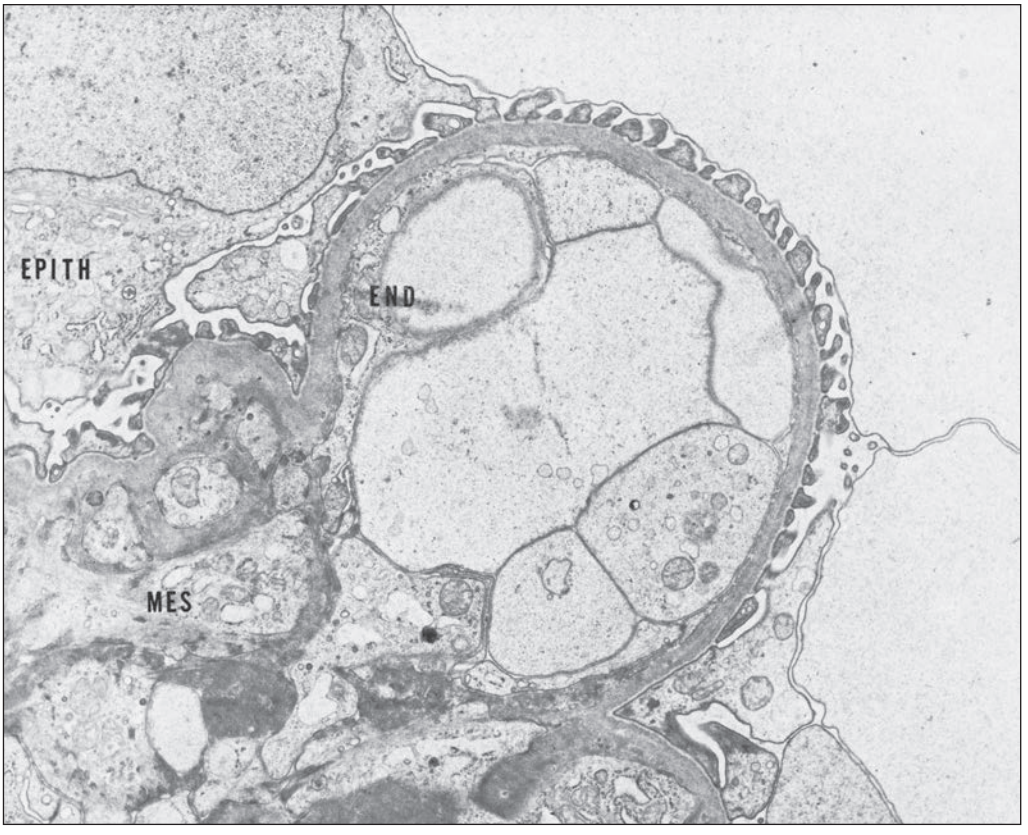
Early studies proposed that subendothelial deposits were derived from fibrinogen (66–73). Demonstration of fibrin or fibrinogen by immunofluorescence further provided support for abnormal intravascular coagulation (80,84) (Fig. 19.15). Immunofluorescence showed IgM and fibrinogen staining of mesangial and glomerular capillary areas and arterioles in most patients (24 of 36), with an additional few patients showing staining with either antisera. Occasional biopsies stained with IgA, IgG, or complement components C3 and C1q, mainly in mesangial areas (44,78,86). Glomerular staining for fibrinogen and fibrin was more pronounced in biopsies taken within the first 2 weeks postpartum, although immunofluorescence positivity and deposits were occasionally observed as late as 2 months after delivery (80,84), with no staining at 3 months in one series (86). The relatively rare dense deposits visualized by electron microscopy support the concept that the immunofluorescence staining represents insudation or hyalin with varying degrees of fibrin-related products rather than immune complexes.

Prognostic Markers

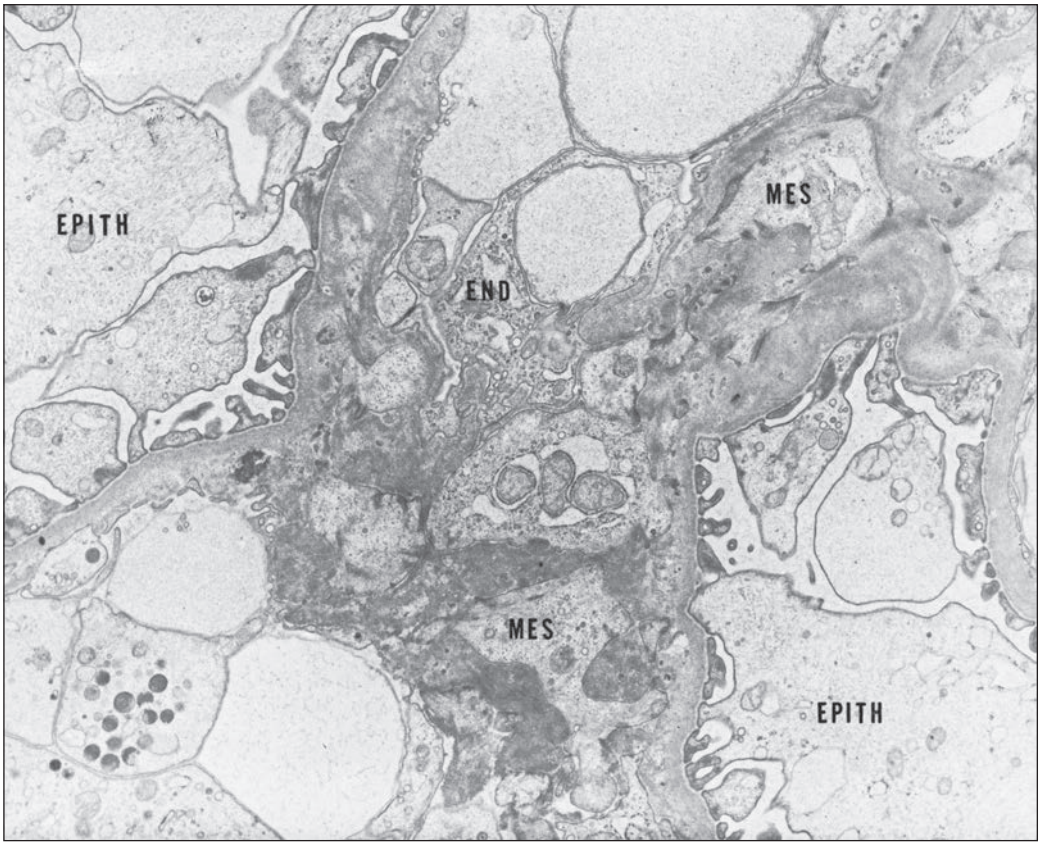
Typical glomerular lesions of endotheliosis are reversible over weeks to months without permanent sequelae. The duration of hypertension after delivery correlated with the severity of glomerular lesions, but not with vascular, that is, arteriolar/arterial, lesions in a series of 20 cases of preeclampsia. All of these patients became normotensive within 3 months, and proteinuria disappeared (87). The presence of additional vascular lesions may signify a worse renal prognosis. Vascular lesions of arteriosclerosis or arteriolosclerosis in patients with preeclampsia are associated with a high incidence of chronic hypertension (see Course and Prognosis) (45).

Focal Segmental Glomerulosclerosis Lesions in Preeclampsia

Idiopathic focal segmental glomerulosclerosis associated with the nephrotic syndrome is discussed in Chapter 6. The effect of this disease on pregnancy and vice versa is discussed under Specific Renal Disease and Pregnancy. However, the lesion of focal and segmental glomerulosclerosis occurs as a manifestation of nonspecific chronic injury in many settings. The significance of this focal and segmental lesion of glomerulosclerosis in pregnancy is controversial. Although the lesion itself has been well described, its relation to preeclampsia or other underlying mechanisms has been debated. Several reports indicate that focal and segmental hyalinosis and glomerulosclerosis may occur de novo during pregnancy with preeclampsia (Fig. 19.16) (53,88–92). These lesions of glomerulosclerosis in pregnancy with preeclampsia resemble those of the cellular lesion of idiopathic focal and segmental glomerulosclerosis. Sclerotic segments of the glomerulus show adhesions with segmentally collapsed capillaries and wrinkled glomerular basement membranes. Overlying podocytes show hyperplasia and frequent vacuoles. Furthermore, some of the subendothelial dense or granular deposits observed in preeclampsia are virtually indistinguishable from the hyalinosis seen in conjunction with idiopathic focal and segmental glomerular lesions in the nephrotic



A



B

FIGURE 19.9 **Electron micrographs from a 19-year-old woman with eclampsia. A:** The capillary lumen is decreased secondary to swelling of the endothelial cell (END). The intercapillary cell mass, including mesangium (MES), appears increased, and there is an increase in amorphous material along the inner surface of basement membrane, especially in the region of the intercapillary cell mass. Epithelial cell (EPITH) changes are mild, except for occasional large blebs showing almost no filamentous matrix or cytoplasmic particulates. **B:** A prominent mesangium separating two areas of highly vacuolated endothelial cells can be seen. (Transmission electron microscopy, ×9000.) (Both courtesy of Ben Spargo, MD.)

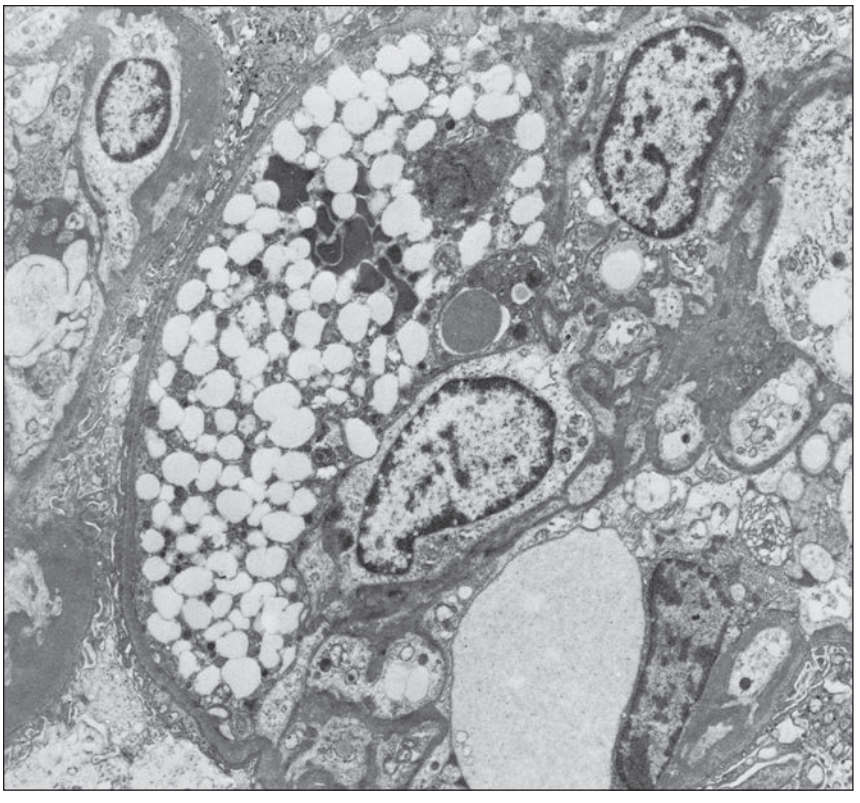


FIGURE 19.10 Electron micrograph shows a glomerular intracapillary cell with numerous empty spaces from an 18-year-old patient with preeclampsia. New basement membrane formation is also apparent. (Transmission electron microscopy, ×4656.)

syndrome. Segmental sclerosis involves appreciable numbers of glomeruli, 28% to 62% in one study and 10% to 50% in another (89,92).

Difficulties arise in establishing a causal link between preeclampsia and biopsy lesions because of the imprecision of the clinical diagnosis of preeclampsia, the variable timing of biopsies, and the lack of prepregnancy or follow-up data. Gaber and Spargo (53) have suggested that lesions other than endotheliosis represent preexisting disease processes other than preeclampsia. These investigators studied renal biopsies performed on average 8 days postpartum from 20 patients with severe preeclampsia characterized by marked hypertension,

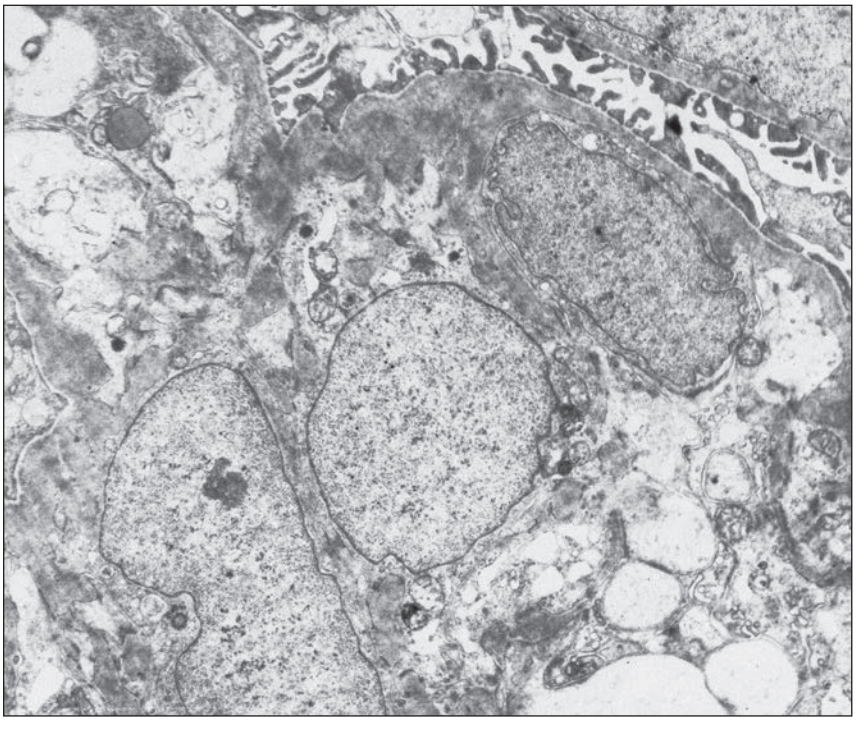


FIGURE 19.11 Electron-dense material on the endothelial side of the glomerular basement membrane (upper part of picture). (Transmission electron microscopy, ×6500.) (Courtesy of Mary MacDonald, MD.)

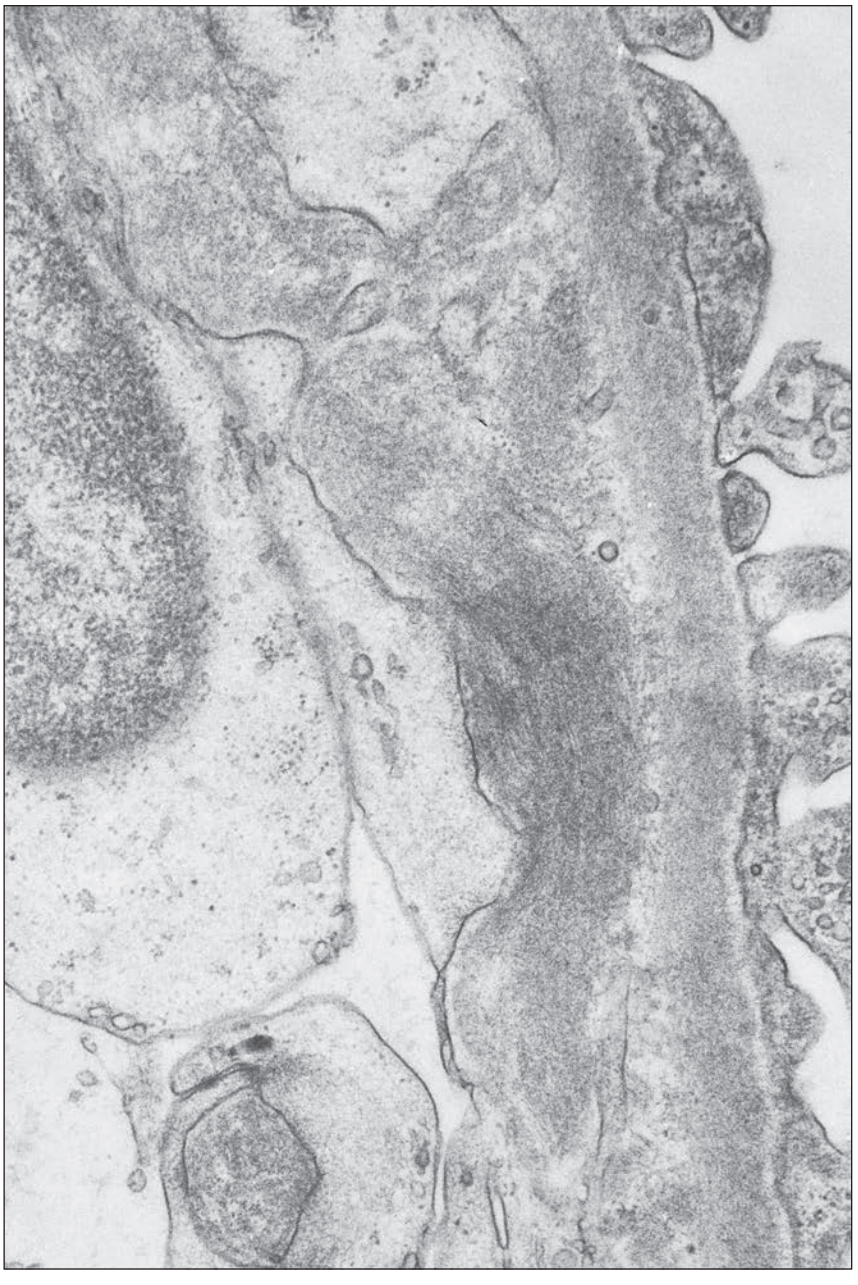


FIGURE 19.12 Fibrillar electron-dense material on the endothelial side of the basement membrane. (Transmission electron microscopy, ×31,000.) (Courtesy of John Lee, MD.)

proteinuria, and edema. Focal segmental sclerosis was present in seven patients, five of whom also had arteriosclerosis. The authors suggested that the focal sclerosis related to underlying nephrosclerosis and not to preeclampsia. They further suggested that the prognosis would be that of patients with preeclampsia with underlying nephrosclerosis, 74% of whom develop persistent hypertension. Unfortunately, no follow-up was reported for the patients in this series to explore this hypothesis further.

In another series of preeclamptic patients with focal segmental glomerulosclerosis (91), 2 of 13 showed frequent hyaline arteriolosclerosis, a lesion that does not itself indicate preexisting nephrosclerosis (72). Milder arteriolar lesions were seen in four additional cases. Vascular lesions were less pronounced in a control group of six patients with typical preeclampsia lesions without segmental glomerulosclerosis lesions. However, seven patients with focal segmental glomerulosclerosis did not have arteriosclerosis, in contrast to the findings of Gaber and Spargo (53). In another study of eight patients with focal segmental glomerulosclerosis and preeclampsia (90), none showed intimal fibroplasia, again supporting the concept that vascular disease does not account for the development of these glomerular lesions. Focal segmental glomerulosclerotic lesions were present in one study of patients with preeclampsia who did not have preexisting hypertension or proteinuria before pregnancy (85,92). Further interesting evidence supports the concept that glomerulosclerosis may be part of the spectrum of injuries induced by preeclampsia. A patient with repeated hypertension during her pregnancies had an initial renal biopsy with only typical changes of preeclampsia and no vascular lesions, followed by a later biopsy with focal segmental glomerulosclerosis (44).

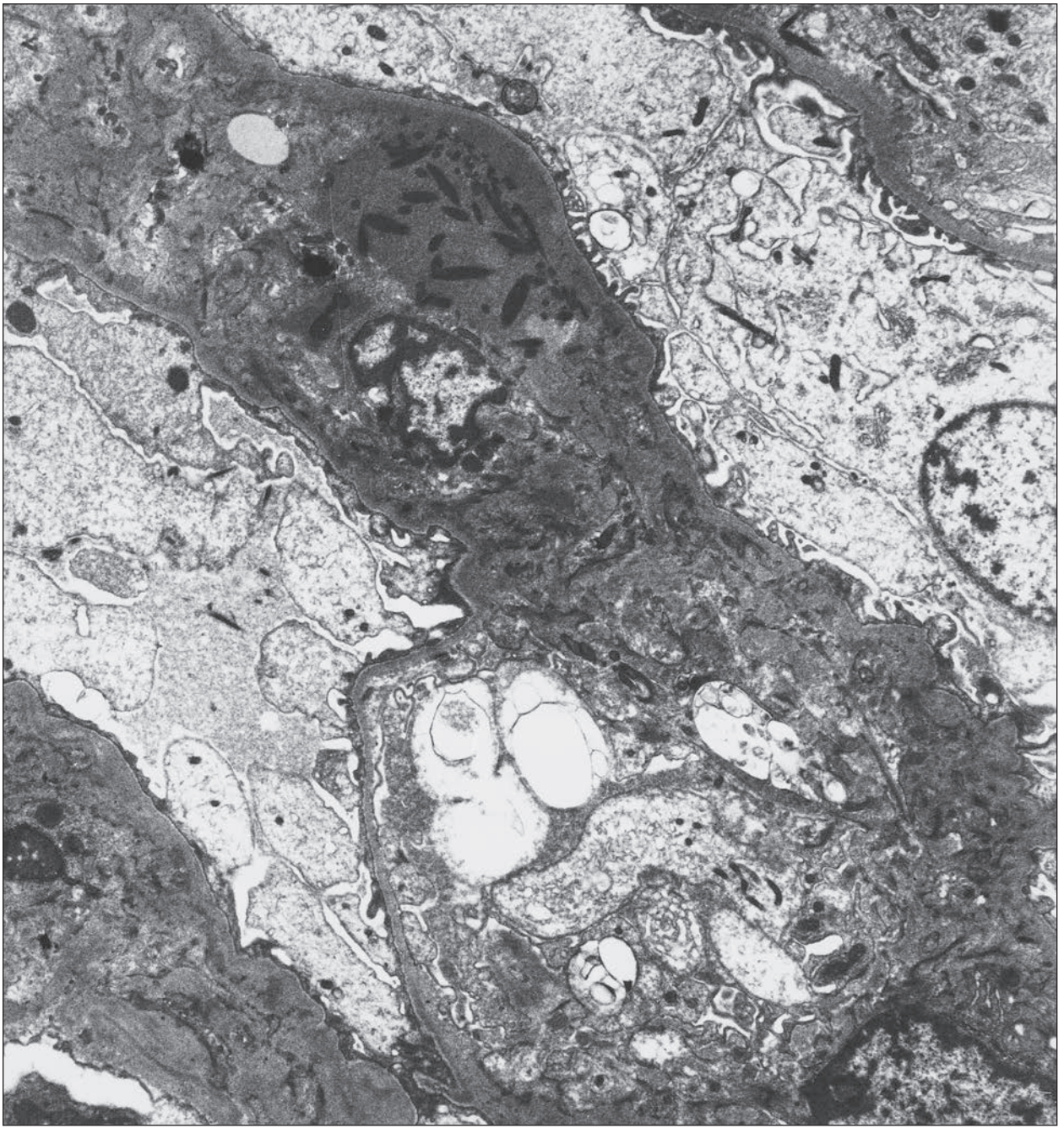
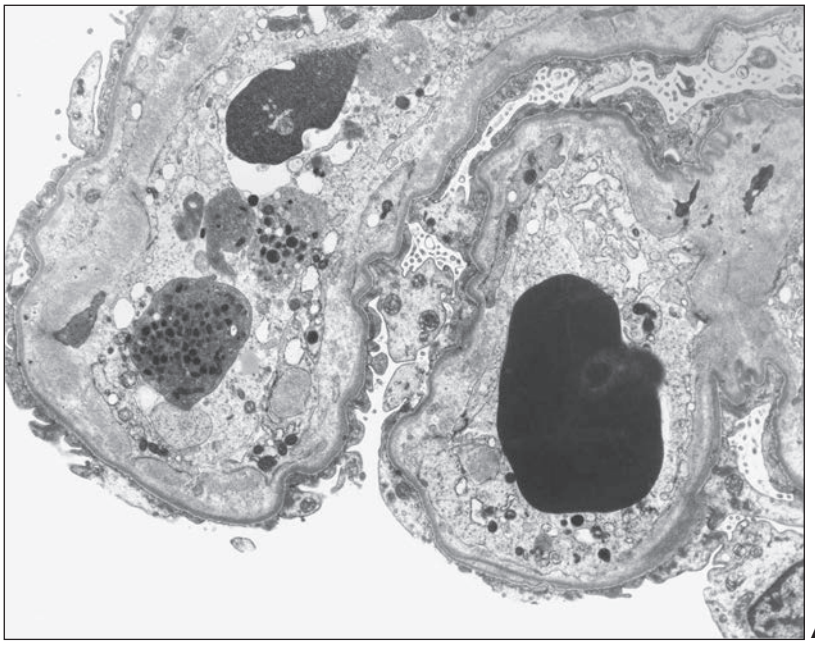


FIGURE 19.13 Mesangial and endothelial cell swelling, subendothelial densities, and fibrin tactoids in a glomerulus from a patient with severe preeclampsia. The biopsy was performed 5 days postpartum. (Transmission electron microscopy, ×8330.)

Patients with preeclampsia and additional focal segmental glomerulosclerosis differ in several respects from those who have only preeclampsia. In a large study, renal biopsies obtained 8 to 10 days postpartum were analyzed (92). In all 42 women with a clinical diagnosis of preeclampsia, the typical morphologic lesions described in Pathologic Changes (p. 821) were present: endothelial cell swelling, double contour of the glomerular basement membrane, and occasional glomerular subendothelial deposition of IgM and fibrin (92). Superimposed focal segmental glomerulosclerosis was present in 19 of these 42 women. The patients in this group more commonly were multiparas and had more severe hypertension, and nearly all presented with the nephrotic syndrome (18 of 19 vs. 5 of 23 among women without these lesions). Despite these findings suggesting more severe injury, all patients had resolution of proteinuria within 3 months of delivery, and only one patient showed persistent hypertension during the follow-up period.

Similar findings were reported in patients from Japan. Only patients with typical preeclampsia clinically in a first pregnancy and without any evidence of preexisting disease were included in this study (91). Renal biopsies were performed on average 18 days after delivery. Typical changes of preeclampsia were present



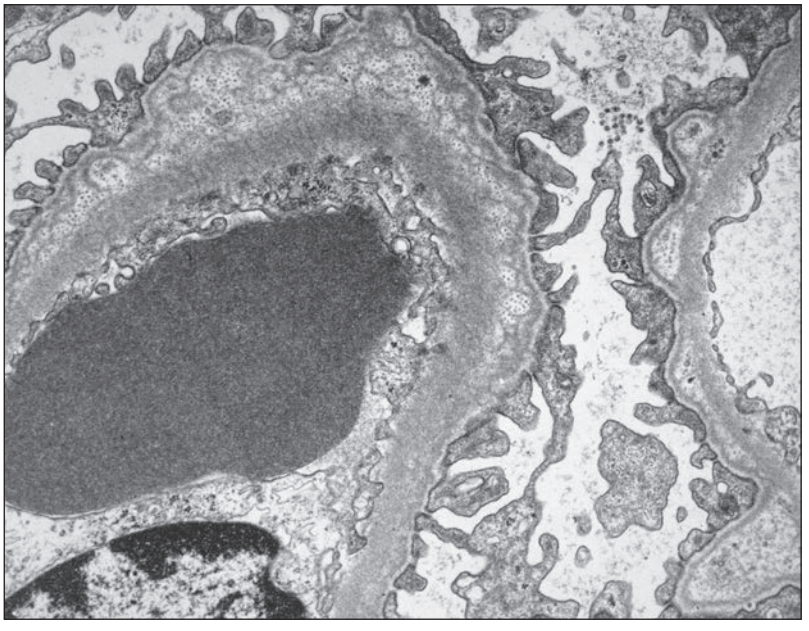


FIGURE 19.14 Glomeruli from patient months after severe preeclampsia. A: The numerous double contours seen by light microscopy are revealed to be due to interposition and increased lamina rara interna. (Transmission electron microscopy, ×4000.) **B:** This patient has marked increased lamina rara interna, which contributes to the split appearance of the GBM by light microscopy. (Transmission electron microscopy, ×7000.) (Both courtesy of Vivette D'Agati, MD.)

in all biopsies: moderate endothelial swelling, circumferential cellular interposition, and increased widening of the lamina rara interna. In addition, focal segmental glomerulosclerosis with occasional hyaline deposits and increased glomerular visceral epithelial cell droplets were observed in 13 of the 19 patients. These lesions involved on average 20.7% of glomeruli (range, 3.1% to 56.5%), the extent of sclerosis correlating with duration of marked proteinuria after delivery. These sclerotic lesions were not restricted to the urinary pole but also occurred at the vascular pole and at intermediate glomerular locations. Proteinuria disappeared in all patients after pregnancy, although it persisted longer in patients with focal segmental glomerulosclerosis (5.7 vs. 2.3 months). One patient with a second biopsy showed less severe glomerular and vascular lesions than in the first biopsy. Remarkably, in three patients, no proteinuria or hypertension developed during a second pregnancy, including the patient with the most extensive glomerulosclerosis. These studies show that focal segmental glomerulosclerotic lesions are not infrequent among preeclamptic women who undergo renal biopsies, and these lesions do not necessarily impart a poor prognosis (Table 19.1). Thus, these patients with preeclampsia and focal segmental glomerulosclerosis did not show the striking increase of hypertension at long-term follow-up seen in patients with nephrosclerosis and superimposed preeclampsia, nor did they show the progressive renal failure typical of idiopathic focal segmental glomerulosclerosis.

Several studies have investigated possible mechanisms contributing to this injury. Factors associated with progressive glomerulosclerosis include abnormal glomerular hemodynamics and abnormal glomerular growth factors that may manifest

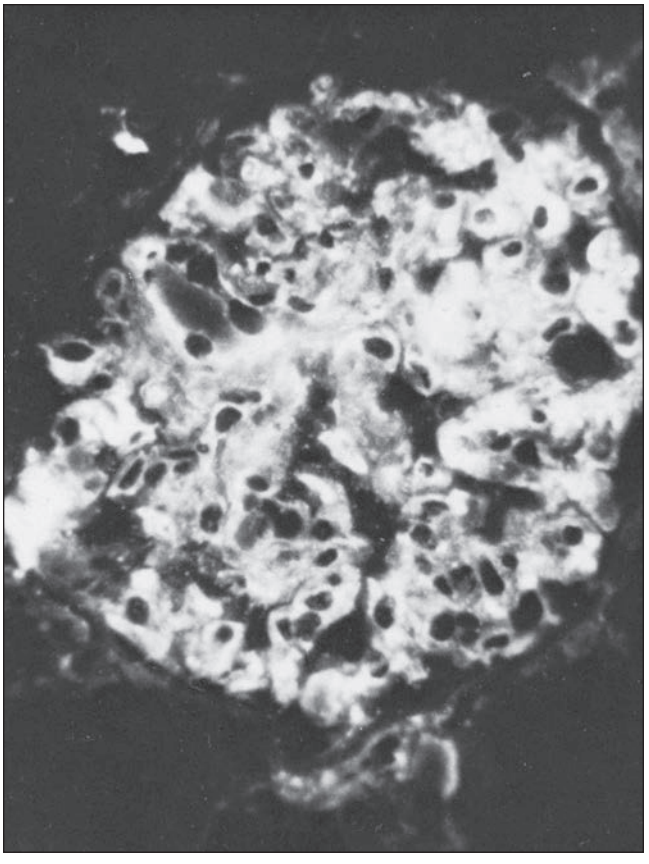


FIGURE 19.15 Glomerulus from a patient with preeclampsia showing positive fluorescence with antiserum to fibrinogen. The distribution is along the inside of the capillary walls. (Courtesy of R. T. McCluskey, MD.)

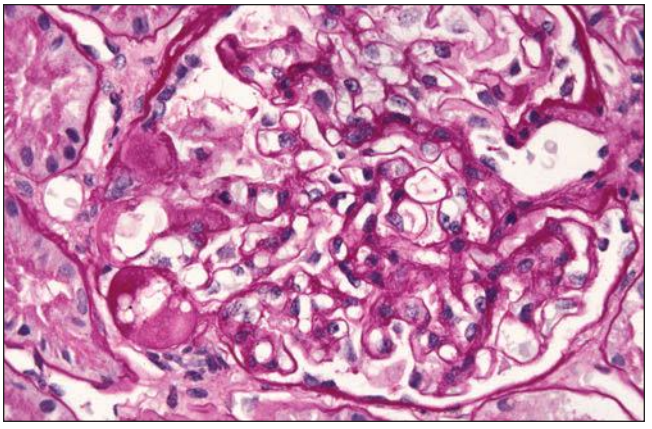


FIGURE 19.16 A patient months after preeclampsia/eclampsia with occasional double contours of the GBM and well-developed segmental sclerosis and hyalinosis. (PAS, ×400.) (Courtesy of Vivette D'Agati, MD.)

as abnormal glomerular enlargement. Morphometric analysis of preeclampsia-associated lesions of focal segmental glomerulosclerosis showed markedly increased glomerular size (74). In contrast, only moderate increase in glomerular size was seen in biopsies from patients with early gestational hypertension or preeclampsia without glomerulosclerosis. In another morphometric study (90), mesangial volume fraction was increased remarkably in patients with classic lesions of preeclampsia and focal segmental glomerulosclerosis compared even with primary focal segmental glomerulosclerosis or normal pregnancy. These data support the concept that abnormal glomerular growth and matrix expansion are prominent in preeclampsia-associated focal segmental glomerulosclerosis.

In summary, although the studies described in this section do not allow definite conclusions regarding the relation of preeclampsia to the focal segmental glomerulosclerosis lesion, some points can be made. The occurrence of apparent de novo focal segmental glomerulosclerosis associated with pregnancy supports a contribution of pregnancy or preeclampsia in their evolution. Furthermore, these focal and segmental glomerulosclerotic lesions in preeclampsia do not represent those of typical idiopathic focal segmental glomerulosclerosis, in which persistent nephrotic syndrome or proteinuria and progression of lesions occur. Most of these patients have a benign outcome at long-term follow-up. In contrast, patients with underlying nephrosclerosis have a high incidence of persistent hypertension after pregnancy (see Course and Prognosis, p. 820). Thus, these findings do not support the concept of underlying nephrosclerosis as a mechanism for the development of glomerulosclerotic lesions in preeclamptic patients. Recent studies show that preeclampsia patients have long-term increased risk for

TABLE 19.1 Frequency and course of glomerular lesions associated with preeclampsia

Lesion	Frequency	Course
Endotheliosis	Invariable	Reversible over weeks to months
Foam cells	Rare peripartum, more frequent postpartum	Resolve
Subendothelial densities	Frequent peripartum	Electron microscopic deposits resolve over first week, Ig staining resolves over 2–3 months
Glomerular basement membrane reduplication	More common in severe disease	Usually resolves rapidly; may persist for months
Fibrin or related products	Rare by light microscopy; frequent by electron microscopy and immunofluorescence	Resolution over weeks
Focal segmental glomerulosclerosis	Variable: 35% (7/20; ref. 53) 45% (19/42; ref. 92) 71% (13/19; ref. 91)	Not clinically progressive

cardiovascular disease, chronic kidney disease, and diabetes mellitus (39). The presence of focal segmental glomerulosclerosis lesions certainly represents more severe acute injury. Possibly, these severe lesions occur selectively in preeclamptic patients with preexisting subclinical renal disease. On the other hand, the increased proteinuria, more severe hypertension, higher incidence of various vascular lesions, and, of course, the focal segmental glomerulosclerosis lesion itself may all represent a more severe manifestation of preeclampsia. The severity of endothelial injury combined with individual susceptibility could contribute to the wide spectrum of glomerular changes seen in preeclampsia. Endothelial injury, altered thrombotic mechanisms, hemodynamic factors, and growth or matrix-promoting factors are altered in preeclampsia-induced injury and could contribute to the evolution of segmental sclerotic lesions in preeclampsia. Whether those with segmental sclerosis associated with preeclampsia have heightened long-term risks for chronic kidney disease beyond those without sclerosis at time of preeclampsia has not been established.

HELLP Syndrome

The HELLP (*h*emolysis, *e*levated *l*iver *e*nzymes, and *l*ow *p*latelet count) syndrome is viewed by some investigators as part of a continuum that includes preeclampsia and hemolytic uremic syndrome (HUS)/thrombotic thrombocytopenic purpura (93,94). This term was initially proposed by Weinstein (95) to describe this catastrophic complication of preeclampsia. The HELLP syndrome may develop rapidly, with progression in hours from an apparently benign condition with minimal abnormalities to a catastrophic illness. Patients may have life-threatening disease with mild (30%) or even absent (20%) hypertension and may not have proteinuria, thus supporting that this entity rather is distinct from preeclampsia (94,96). The incidence of HELLP syndrome among patients with preeclampsia ranges from 4% to 18.9%, with about one to two women affected per 1000 pregnancies (96,97). Patients usually present in the third trimester, but the syndrome may occur earlier or even postpartum (96). Patients manifest with nonspecific malaise, right upper quadrant pain, nausea, vomiting, and headache, or they may have various degrees of jaundice, gastrointestinal and gum bleeding, and hematuria. Laboratory findings are defined by the syndrome's acronym. Laboratory criteria include hemolytic anemia with bilirubin $\geq$1.2 mg/dL and LDH greater than 600 U/L, increased transaminases (aspartate aminotransferase greater than 70 U/L), and decreased platelets (less than 100,000/mm^3). A possible role for complement dysregulation in HELLP syndrome and preeclampsia has been suggested but not proven (98,99).

Prognosis

This condition is potentially life threatening because of the marked coagulation abnormalities, hemolysis, and serious abnormalities of liver function. Recent studies demonstrate maternal mortality of approximately 1% (96). With prompt termination of the pregnancy, the maternal prognosis is favorable (2,93,94). Most patients normalize the abnormal laboratory parameters by day 6 after delivery (96). In a large series of 442 pregnancies with HELLP syndrome, maternal mortality was 1.1%. Perinatal mortality ranged from 7.7% to 60%, depending on the severity of the maternal complications and the degree of prematurity, and was increased in pregnancies with acute kidney injury (97,100).

Long-term prognosis after HELLP syndrome with resulting acute kidney injury was studied in 32 patients. Women with postpartum HELLP syndrome had higher incidence of acute kidney injury than those presenting antepartum: 12% versus 5% (94). A clinical diagnosis of acute tubular injury was made in 31 patients, with cortical necrosis presumed in 1 patient with persistent anuria. In subsequent pregnancies in 8 of the 23 normotensive survivors, only 1 case of preeclampsia occurred, and none showed evidence of renal dysfunction at follow-up. In contrast, the hypertensive survivors frequently developed severe preeclampsia in subsequent pregnancies. One woman developed full-blown HELLP syndrome again, whereas a second patient developed renal failure in a later pregnancy. Two of the five hypertensive survivors required chronic dialysis (94). In more recent series, 10% to 46% of women with HELLP syndrome and acute kidney injury needed acute dialysis, but most showed recovery of kidney function (96). These data also indicate a poorer prognosis for subsequent pregnancy when HELLP syndrome occurs with preexisting hypertension.

Etiology and Pathogenesis of Preeclampsia and Eclampsia

General

Numerous mechanisms have been postulated to contribute to the development of preeclampsia/eclampsia. We will focus on those related to key angiogenic factors, including vascular endothelial growth factor (VEGF) and endoglin, and angiotensin type 1 (AT1) receptor agonistic antibodies, and how these mechanisms may interact with placental alterations in preeclampsia.

Placental Mechanisms

Placental vascular abnormalities were recognized over 70 years ago in preeclampsia and eclampsia (101). Several obstetric conditions, such as hydatidiform mole, multiple-gestation births, and hydrops, and medical conditions, such as diabetes and hypertension, are associated with increased risk of preeclampsia. A possible common underlying feature is placental hypoperfusion related to underlying vascular injury. In normal pregnancy, cytotrophoblasts invade the endometrium and myometrium and differentiate into syncytiotrophoblasts and extravillous trophoblasts and penetrate the spiral arteries. The endothelial lining and most of the muscle layer of these spiral arteries are destroyed, contributing to their capacity to vasodilate during gestation, thus establishing uteroplacental circulation by 12 to 13 weeks' gestation. This remodeling process is dependent on phenotypic switch of cytotrophoblasts, a process referred to as *pseudovasculogenesis* (102). These changes result in marked increase in blood flow to the fetus and allow the fetal trophoblasts to be bathed in maternal blood. This cytotrophoblast invasion depends on complex cell-cell and cell-matrix interactions. Preeclampsia is associated with abnormal differentiation of cytotrophoblasts and altered expression of integrins, key adhesion molecules (103). The end result is faulty cytotrophoblast invasion of the decidua, with resulting shallow invasion that breaches fewer arterioles. Reduced trophoblastic invasion may be a pivotal injury leading to the cascade of events culminating in full-blown eclampsia. This initial failure to invade results in a hypoxic environment that further inhibits cytotrophoblast differentiation and invasion, thus perpetuating a deleterious feedback mechanism that sets the stage for

later pregnancy complications such as preeclampsia (104). The result is acute atherosis of the placenta, which is characterized by fibrin deposition along the intima and fibrinoid necrosis and foam cell invasion of the media of the spiral arteries. These lesions lead to thrombosis and infarctions of and reduced blood flow to the uterus and placenta.

Endothelial injury is the most constant morphologic finding in renal biopsies of preeclampsia. Injured endothelium can result in altered elaboration of or sensitivity to vasoactive substances, increased thrombotic activity, and vascular permeability. Normal pregnancy is a condition with enhanced thrombotic and fibrinolytic activities, which typically return to normal prepregnant states within an hour after placental delivery (105). This balance of thrombosis and fibrinolysis is shifted further toward thrombosis in preeclampsia. Fibrin degradation products and beta-thromboglobulin are increased and thrombin activity is enhanced, whereas antithrombin levels are increased and platelets are decreased (105,106). Fibronectin and factor VIII antigen are released from injured endothelial cells, and their blood levels are also increased in preeclampsia (105). Syncytiotrophoblast microparticles, that is, 100- to 200-nm subcellular particles, and nanoparticles, so-called exosomes, that is, 20- to 40-nm cellular particles, are increased in the circulation in preeclampsia. These microparticles are also qualitatively different from those seen in normal pregnancy in that they can induce inflammation (107). A cascade of endothelial injury initiated by the injured trophoblasts has been hypothesized. The injured trophoblasts are proposed to release a factor into the systemic circulation that induces sublethal endothelial cell injury, resulting in low-grade intravascular coagulation, loss of normal permeability barrier, and increased sensitivity to pressors, culminating in the clinical syndrome of preeclampsia (105,108). Studies of cytotoxic effects of serum from preeclamptic women support this hypothesis: Cultured human endothelial cells showed greater injury when exposed to sera from preeclamptic women than sera from the same patients within 48 hours after delivery or from normal women before or after delivery (104). Key angiogenic factors normally released from the placenta, including soluble form of the VEGF receptor, sFlt-1, placental growth factor (PlGF), and endoglin, may be the key elusive intermediary mechanisms linking placental and renal injuries in preeclampsia/eclampsia (109–112).

Angiogenic Factors in Preeclampsia

Vascular endothelial–derived growth factor-A (VEGF-A) is a primary growth factor for endothelial cells and in the glomerulus is produced exclusively by the podocytes (113). Vascular factors such as VEGF and the angiopoietins are essential for glomerular endothelial cell growth, and VEGF is needed for maintenance of fenestrae (114,115). Thus, the kidney might be particularly susceptible to loss of VEGF, as the fenestrated endothelium is essential for normal glomerular function. VEGF is also implicated in the vascular remodeling that occurs when cytotrophoblasts convert from an epithelial- to an endothelial-type phenotype and invade maternal spiral arteries. This process is defective in preeclampsia, and the resultant ischemia is proposed to induce systemic endothelial dysfunction (112). The main receptor for VEGF, fms-like tyrosine kinase-1 (Flt-1), exists in membrane-bound and soluble forms (sFlt-1). The soluble form of the receptor lacks a cytosolic domain and functions as an antagonist of VEGF and placental growth factor (PlGF), decreasing the free amounts of these factors available to transduce active signaling via intact membrane-bound Flt-1. Levels of sFlt-1 increase during the last 2 months of normal pregnancy. However, in preeclamptic women, sFlt-1 increases earlier, preceding the clinical onset of preeclampsia (109). A splice variant of sFlt-1, sFLT-1-14, is particularly potent in inhibiting VEGF. sFlt-1 increase in the circulation is linked to increased placental expression, particularly in areas of degenerative syncytiotrophoblasts, so-called syncytial knots (33). Indeed, this increase of sFlt-1 in preeclamptic patients was associated with decreased circulating levels of free VEGF and PlGF, to levels that may be below those required to maintain normal endothelial function. Experimental evidence proved the causality and clinical relevance of these alterations. Exogenous administration of sFlt-1 to pregnant rats induced hypertension, proteinuria, and glomerular endotheliosis, the classic lesion of preeclampsia (110). Additional studies using podocyte-specific manipulation of VEGF-A showed that deletion of just one VEGF allele resulted in proteinuria and endotheliosis in mice. Further, neutralization of VEGF with either exogenous sFlt-1 or antibody caused proteinuria in mice. These studies thus demonstrate specifically that relative lack or inhibition of VEGF could result in proteinuria and endotheliosis lesions (114).

However, not all women with high sFlt-1 or low PlGF levels develop preeclampsia. Additional circulating factors that potentially affect endothelial cell function in preeclampsia have been identified. Endoglin is a receptor for transforming growth factor-β1 (TGF-β1) and TGF-β3 and is expressed in endothelial cells and in syncytiotrophoblasts (113). Endoglin localizes to caveolae and is linked to nitric oxide synthase activation. Soluble endoglin is increased in the serum in the last 2 months of normal pregnancy but with earlier and higher increases in preeclampsia (113). Increased soluble endoglin may inhibit TGF-β1 signaling and nitric oxide activation and thus impair normal endothelial cell function. Ratios of these various angiogenic modulators have been assessed as predictors of preeclampsia. An increase in sFlt-1:PlGF ratio predicted preeclampsia. Increased soluble endoglin preceded an increase in this ratio (113). An algorithm incorporating serum free PlGF and various clinical parameters, including blood flow measures, had high sensitivity and specificity (94.1% and 94.3%, respectively) in predicting preeclampsia (116). Increased podocyte loss in urine is also seen early in preeclampsia and may reflect early glomerular injury (117). Renal biopsies from preeclamptic patients indeed show less staining for the key podocyte markers, nephrin and synaptopodin (118). However, the lack of easy availability of all these measures could limit utility. Importantly, the key initiating events that could up-regulate sFlt-1 expression in the placenta and change other angiogenic modulators in preeclampsia have not been determined.

Renin-Angiotensin System

The renin-angiotensin system has long been studied in diseases manifesting hypertension, but observations that plasma renin activity and angiotensin II levels frequently are not increased in preeclampsia had led to less enthusiasm for a primary role of this pathway in its pathogenesis. However, many, albeit not all, preeclamptic patients have an agonistic IgG autoantibody that stimulates the AT1 receptor, presumably by altering conformation of the receptor and increasing its ability to bind circulating angiotensin II (119–121). The possibility of an interaction of

AT1 receptor agonistic autoantibodies in contributing to this initial ischemic step is interesting but has not been proven. The AT1 receptor transduces classic angiotensin actions, including vasoconstriction, aldosterone secretion, and growth and matrix synthesis. In addition to these agonist antibodies to the AT1 receptor, AT1 receptors may dimerize or heterodimerize with the bradykinin B2 receptor, resulting in enhanced angiotensin II responsiveness (122,123). The AT2 receptor, which counteracts many AT1 receptor actions, was decreased in one study of pregnancy-induced hypertension, supporting an imbalance of AT1/AT2 receptors in this condition (124). Further, the counteracting angiotensin metabolite, Ang (1–7), was decreased, as was the angiotensin-converting enzyme (ACE) 2 level in preeclampsia, supporting a deranged renin-angiotensin system in the pathogenesis of preeclampsia. AT1 receptor agonistic antibodies from preeclamptic patients infused into pregnant mice resulted in glomerular lesions of preeclampsia. These lesions were prevented by treatment with an angiotensin receptor blocker or by a neutralizing peptide (125). When recombinant VEGF121 was added to this AT1 agonistic antibody-induced preeclampsia model, disease was prevented (126). However, AT1 agonistic antibodies are also detected in serum in malignant hypertension and in humoral rejection, so this mechanism may not be specific for preeclampsia (32,127). Unfortunately, the teratogenic effect of angiotensin inhibitors, either ACE inhibitors or angiotensin receptor blockers, limits their use as therapeutic agents in preeclampsia. The presence of the AT1 agonistic autoantibody was also linked to increased secretion of plasminogen activator inhibitor-1, PAI-1, a key modulator of fibrinolysis and proteolysis through its actions to inhibit tissue-type and urokinase-type plasminogen activators (t-PA, u-PA) (128). PAI-1 is increased in normal pregnancy, but increased even further in patients with preeclampsia, correlating with severity of placental damage (129). PAI-1 could also theoretically affect trophoblast invasion by preventing proteolysis and fibrinolysis (129). Interaction of these AT1 agonistic antibodies with the VEGF system has also been suggested. Thus, VEGF-mediated angiogenesis can be decreased with AT1 receptor blockers (120,130). The specific events that stimulate such autoantibodies have also not been defined (111).

Other Vasoactive Substances

Vasoactive substances are altered in preeclampsia. Although women with normal pregnancies show refractoriness to the pressor effects of infused angiotensin II, in preeclampsia, the responsiveness is more similar to, or even increased over, that of nonpregnant women, perhaps reflecting agonistic AT1 autoantibodies (see above) (131). Other vasoactive substances may also contribute to the abnormal vasoconstriction in preeclampsia. Despite decreased plasma volume in preeclampsia, plasma atrial natriuretic peptide levels are elevated. Furthermore, women with preeclampsia have reduced prostacyclin and thromboxane excretion. In a study of severe preeclampsia, these mediators were correlated inversely with plasma creatinine and plasma renin (131). Endothelin levels were reported increased in women with preeclampsia and were correlated inversely with creatinine clearance (132). However, another study failed to show increased endothelin activity in preeclampsia, and placental tissue mRNA levels for endothelin precursors were not increased compared with those in normal pregnancy (133). Additional studies have shown decreased density of umbilical artery dopaminergic receptors in preeclampsia, possibly contributing to impaired dopaminergic vasodilatory tone (134). Animal models show a potential role of catechol-*O*-methyltransferase (COMT), which converts 17-hydroxyestradiol into 2-methoxyestradiol. This compound in turn inhibits hypoxia-inducible factor 1-α, which appears to have antiangiogenic activity (33). Decreased placental COMT and plasma 2-methoxyestradiol in preeclampsia have been observed. Functional polymorphisms of the COMT gene have been suggested to contribute to preeclampsia risk (33).

Immune Mechanisms

Previously, immune mechanisms were sought in preeclampsia. Glomerular and vascular IgM deposits are present, as discussed in Pathologic Changes (p. 825). Circulating soluble immune complexes are not detected in preeclamptic patients (43). However, recent data show an abnormal persistence in preeclampsia of decidual natural killer (dNK) cells, which normally invade the decidualized uterus before implantation. dNK cells express angiogenic factors including VEGF, PlGF, and angiopoietin-2. The dNK cells and macrophages normally infiltrate and disrupt the vascular smooth muscle cell layers of the spiral arteries, which may influence or prime for subsequent remodeling by invading trophoblasts. Trophoblasts express Toll-like receptors and may thus directly interact with macrophages and other immune components (107). In preeclampsia, dNK cells remain increased in the decidua and are associated with increased interferon-γ and tumor necrosis factor–α levels (33). These observations provide potential targets for modulation of the key placental abnormalities that occur in preeclampsia, but the specific mechanisms or potential contribution of innate immunity in preeclampsia remain unknown.

Genetic Factors

The risk of preeclampsia is increased in nulliparous women with an affected mother or sister, fourfold and sixfold, respectively (107,135). This familial aggregation of preeclampsia has suggested a contribution of genetic factors. Interestingly, the risk of preeclampsia was increased in offspring from either men or women who were the product of a pregnancy complicated by preeclampsia, suggesting both maternal and paternal genetic contributions (135). Inheritance patterns of classic preeclampsia are consistent with a recessive autosomal single gene, with a calculated gene frequency of 0.2% to 0.25%. Underlying genetic factors likely also contribute to increased risk of preeclampsia in patients with underlying hypertension of renal disease. However, these conditions likely reflect polygenic traits for which precise risk and genetic basis are difficult to define. Studies of mutations of the renin-angiotensin system genes have previously shown linkage of polymorphisms of the angiotensinogen or ACE gene to preeclampsia in some patients. These polymorphisms may have direct functional consequences to increase renin-angiotensin system activity. However, recent studies and analyses have failed to confirm such associations (136).

Numerous genes involved in the regulation of blood pressure or coagulation have been investigated to search for polymorphisms or mutations in preeclamptic women, without any conclusive evidence of such an effect (see above). However, a locus on chromosome 2p13 showed significant association with preeclampsia in a genome-wide scanning study of Icelandic families, confirmed in a study of patients from New Zealand and Australia (112). This site was distinct from that associated with linkage with the HELLP syndrome, localized

to chromosome 12q. Importantly, this locus is distinct from the Flt-1 locus at 13q12. The gene for sFlt-1 is on chromosome 13 (137). Of note, mothers carrying fetuses with trisomy 13, but not those with trisomy 18 or 21 fetuses, show a higher incidence of preeclampsia than other trisomies and have higher levels of sFlt-1, suggesting that overexpression of Flt-1 could be caused by trisomy 13 in these patients.

ACUTE KIDNEY INJURY IN PREGNANCY

The incidence of pregnancy-related acute kidney injury varies worldwide according to the level of obstetric care. In industrialized countries, acute kidney injury has become rare, occurring in an estimated 1 in 20,000 deliveries (138). In contrast, in South Africa, despite advances in care, the incidence remains high in the indigent population. In 1978, acute kidney injury occurred in 1 in 450 deliveries, improving to 1 in 1000 in the 1990s (139), primarily because of a decrease in septic abortions in that country (see Septic Abortion, below). Similar prevalences of pregnancy- and abortion-related acute renal failure were reported from India and Argentina (140).

Acute kidney injury may occur for any reason during pregnancy, including reasons not specific for pregnancy (141). Acute kidney injury in the first trimester is usually due to prerenal causes, most commonly due to hyperemesis gravidarum, or due to septic abortion. In late pregnancy and the peri/postpartum period, obstetrical causes of hemorrhage dominate as etiologies of acute kidney injury (96). Acute kidney injury may also be due to preeclampsia, acute fatty liver of pregnancy, HUS, or sepsis. Among 57 pregnant women with acute kidney injury, causes included acute pyelonephritis, other infections, severe preeclampsia or eclampsia, abruptio placentae, prolonged intrauterine fetal death, uterine hemorrhage, and, in a small group, postpartum idiopathic acute kidney injury (142). Pregnancy may also result in nonglomerular complications leading to acute kidney injury, such as acute hydronephrosis presumed to result from obstruction. Spontaneous rupture of the renal pelvis has been reported (143). No specific site of obstruction was delineated, nor were calculi demonstrated.

This section is a discussion of some of the main causes of acute kidney injury in pregnancy:

- Acute pyelonephritis
- Preeclampsia, eclampsia, and HELLP syndrome
- Uterine hemorrhage
- Septic abortion
- Cortical necrosis
- Acute fatty liver of pregnancy
- Pregnancy-associated thrombotic microangiopathy

Acute Pyelonephritis

Acute pyelonephritis (see Chapter 24) is relatively common in pregnancy, affecting approximately 1% to 2% of gravidas (141), and it may cause a transient decrease in the GFR (144). Septic shock, and not pyelonephritis itself, underlies the rare occurrence of acute kidney injury after acute pyelonephritis in pregnancy. In one series of acute kidney injury in pregnancy (142), only 2 of 57 patients had underlying acute pyelonephritis.

Preeclampsia, Eclampsia, and HELLP Syndrome

Acute kidney injury is a rare complication of severe preeclampsia or eclampsia, possibly reflecting early recognition and treatment of preeclampsia in most cases. Patients with preeclampsia or eclampsia who developed acute kidney injury tended to be multiparas and therefore also older than other preeclamptic patients (142,145). When acute kidney injury in preeclamptic patients resulted in death, arteriolar and arterial changes were frequent (146). In a series of 154 patients with well-defined eclampsia, no cases of acute kidney injury were found; further suggesting that acute kidney injury is rare in pure preeclampsia or eclampsia (147). The acute kidney injury appears to result from acute tubular injury or extensive cortical necrosis. The underlying mechanism is thought to be ischemia, which may be contributed to by glomerular occlusion by endotheliosis or intravascular coagulation. Coexistence of tubular necrosis and glomerular changes of preeclampsia has been reported (24). Patients with HELLP syndrome and hemorrhage are at particular risk of ischemia and resultant organ damage, including kidney injury. The risk of acute kidney injury is higher if HELLP syndrome develops postpartum (93,94). Acute kidney injury occurs in as many of 8% of pregnancies in patients with HELLP syndrome. About 10% to 46% of these women with HELLP syndrome and acute kidney injury required dialysis acutely, but most recovered renal function (96) (see HELLP Syndrome, p. 832).

Uterine Hemorrhage

Acute kidney injury is especially common in pregnancy complicated by abruptio placentae and disseminated intravascular coagulation. Hemorrhage was implicated as a cause underlying acute kidney injury in 7% of patients in one series from France (142) and in up to 58% to 79% of acute kidney injury cases in pregnancy from Great Britain and India, respectively (148,149). Patients with preeclampsia may be especially susceptible to acute kidney injury in response to hemorrhage (141,148). The mechanisms are unknown, but conceivably they relate to altered volume homeostasis and pressor sensitivity in preeclampsia.

Septic Abortion

In contrast to the low incidence of pregnancy-related acute kidney injury in developed countries, the incidence in developing countries remains astonishingly high, mainly because of complications of septic abortion. In France, a decline in acute kidney injury cases associated with abortion from 18.6% to 0.6% occurred over a 13-year span beginning in 1966 (150). In South Africa (139), acute kidney injury occurred in 1 in 450 deliveries in 1978, improving to 1 in 1000 in the 1990s, associated with a marked decrease in acute kidney injury cases caused by septic abortions from 65% to 29%. In a series of cases of pregnancy-related acute kidney injury from India (148), 60% followed abortion. Most pregnancy-related acute kidney injury resulted from septic abortion in Argentina as well; only 79% survived, with one survivor developing chronic kidney disease (141). Although most of these patients had acute tubular necrosis as the proximal cause of acute kidney injury, some developed cortical necrosis (see below). The change in incidence over time of pregnancy-associated acute kidney injury was assessed in a region of India. The overall incidence of all acute kidney injury in pregnancy fell from 15% in 1982–1991 to 10% in 1992–2002, with a trend for decrease abortion and puerperal sepsis–associated acute kidney injury (151).

Cortical Necrosis

In developing countries, acute renal cortical necrosis more commonly underlies acute kidney injury than in the Western world. Furthermore, over half of the cases of acute cortical necrosis were related to pregnancy, a finding that perhaps reflects the high risk of bilateral cortical necrosis with acute kidney injury in pregnancy (150). These findings are in contrast to a marked decline in acute cortical necrosis, in particular that resulting from obstetric causes, in Western countries, from 1 in 10,000 from 1961 to 1970 to less than 1 in 80,000 over the next 10 years in a series from Ireland (152). Cortical necrosis observed associated with pregnancy in a region of India decreased from 17% in 1982–1991 to 2.4% after 2000 (151).

The highest incidence of bilateral cortical necrosis occurs after abruption of the placenta or prolonged intrauterine fetal death (142). Bilateral renal cortical necrosis is also relatively frequent among patients with postpartum renal failure (21%), in contrast to only 1.5% in postabortion acute renal failure. In a series of 113 patients with tissue-proven diagnosis of cortical necrosis from India (140), 37% of cases were associated with complications with late pregnancy and 20% followed septic abortion. Recovery of renal function may continue up to the 3rd year (142).

Acute Fatty Liver of Pregnancy

Acute fatty liver of pregnancy is a rare complication of late pregnancy that may be fatal. The incidence is estimated to be from 1 in 7000 to 1 in 20,000. The underlying cause is an autosomal recessive gene defect in long-chain 3-hydroxyacyl-CoA dehydrogenase, an enzyme that is key for mitochondrial fatty acid β-oxidation (96). The excess fatty acids from the fetus reach the maternal circulation and are deposited in the liver and overwhelm mitochondrial capacity, thus resulting in liver injury. Patients develop fever, nausea, vomiting, abdominal pain, and jaundice. The liver shows swollen hepatocytes with microvesicular fat. Maternal and fetal deaths were common, although more recent advances in early recognition and treatment have improved maternal survival dramatically (153). Renal failure is typically not severe, although it occurs commonly (60%) (96,142). In severe cases, progressive encephalopathy, coagulopathy, acute renal failure, adult respiratory distress syndrome, and pancreatitis may develop.

Renal biopsies have shown fatty vacuolization in renal tubules and findings of tubular regeneration or focal necrosis. Intraglomerular thrombi have been reported rarely (154). Most women have complete recovery of liver and kidney abnormalities after delivery. However, the mortality acutely is 10% to 20% (96).

Pregnancy-Associated Thrombotic Microangiopathy

Clinical Findings

Women with underlying complement dysregulation or ADAMTS13 deficiency may have atypical HUS episodes triggered by pregnancy. These causes of HUS are discussed in further detail in Chapter 18. After a flu-like syndrome, severe acute kidney injury and hypertension develop rapidly, often associated with microangiopathic hemolytic anemia. Some patients have preceding preeclampsia, whereas others have had normal pregnancies. The estimated incidence is 1 in 25,000 pregnancies (155). Disease developed peripartum in about two thirds of patients, and earlier in pregnancy in the remainder. Counihan and Doniach (156) were the first to recognize the association of hemolytic anemia with postpartum renal failure. Postpartum HUS has been described in association with circulating lupus anticoagulant and anticardiolipin antibodies. In one case, termination of pregnancy was followed by marked increase of anticardiolipin antibodies, severe hypertension, and renal failure associated with microangiopathy (157). Patients with pregnancy-associated atypical HUS had severe renal disease, with 81% requiring dialysis, and 62% reached end-stage renal disease within months of onset of disease. A high prevalence of abnormalities in complement regulatory genes was identified. Renal biopsies showed typical thrombotic microangiopathy lesions (158). Patients in an older series with lesions limited to glomeruli showed a better prognosis than when larger arteries were involved, as in other causes of HUS (159). Clinical diagnosis of HUS cannot be based solely on the presence of schistocytes, because these may occur in other pregnancy-associated conditions, including preeclampsia and eclampsia (160). Furthermore, biopsies performed during pregnancy at times of deterioration of renal clinical parameters showed fibrin thrombi regardless of underlying renal disease (IgA nephropathy, reflux nephropathy, or focal sclerosis with hyalinosis). These lesions were not associated with clinical features of preeclampsia in these patients, and they are postulated to heal to form a lesion of segmental hyalinosis (79). Thus, the diagnosis of the HUS cannot be based solely on the presence of fibrin thrombi.

Microscopic Findings

Glomeruli

Endothelial cell swelling and intraglomerular fibrin thrombi occur, consistent with thrombotic microangiopathy (see Fig. 19.6B; see Chapter 18 for a full discussion) (142). When severe vascular lesions, such as extensive thrombi, are present, glomerular ischemic changes predominate.

Tubules

The kidney structure may be within normal limits, or focal tubular necrosis, tubular regeneration, or even cortical necrosis may be present.

Vessels

Vascular changes suggestive of nephrosclerosis, that is, intimal fibrosis and medial hypertrophy, have been noted in addition to lesions consistent with thrombotic microangiopathy. Interlobular arteries show marked intimal thickening and occlusion by fibrin thrombi. In other cases, lesions similar to those of malignant hypertension or scleroderma have been described (Figs. 19.16 and 19.17) (161,162).

Electron Microscopic Findings

Changes are those seen in other forms of HUS and scleroderma and are thought to reflect a response to intravascular coagulation. There is widening and lucency of the lamina rara interna with an expanded zone with finely fibrillar or finely particulate electron-dense material (Fig. 19.18). Interposed cells may also be present, but there are no electron-dense immune complexes.

Immunofluorescence Findings

Findings are similar to those in other forms of HUS. Fibrinogen and fibrin are typically present, with some biopsies showing staining with IgG and IgM and complement in vessels. Glomeruli typically are negative (159,163,164).

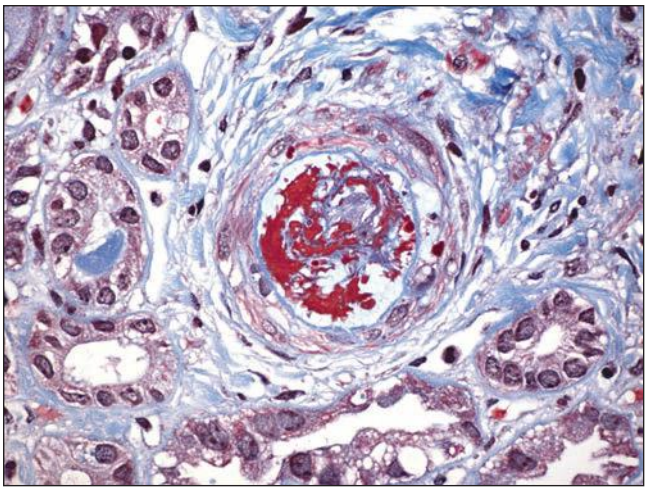


FIGURE 19.17 Interlobular artery in patient after preeclampsia revealing intraluminal fibrin and early intimal proliferation. (Masson trichrome, ×400.) (Courtesy of Vivette D'Agati, MD.)

PREGNANCY AND PREEXISTING RENAL DISEASE

The effect of pregnancy on preexisting renal disease varies, depending on both the type of disease and its severity at conception (165). Similarly, the effect of renal disease on pregnancy outcome is determined both by disease-specific factors and by factors related to the degree of renal dysfunction. Numerous studies have examined whether pregnancy adversely affects the natural course of underlying primary renal diseases and whether pregnancy outcome is influenced by this disease process, as reviewed by Jungers and Chauveau (166). Based on experimental data suggesting an adverse effect of abnormal hemodynamics on renal function, investigators have postulated that the increased renal plasma flow and glomerular filtration during pregnancy could exacerbate the course of renal disease (see Functional Changes in Pregnancy, p. 816). Whether pregnancy changes the natural history of various renal diseases is difficult to ascertain because the variable and slow course of many progressive renal diseases makes it difficult to use patients as their own controls. Few reported series have concurrent control populations, and this limitation adds to the difficulty in determining the effect of pregnancy on renal outcome. In a large controlled series of 148 women with various biopsy-proven renal diseases who were pregnant, the risk of developing chronic kidney disease was not increased compared with the control group of women with similar glomerulonephritides who did not become pregnant (167). Patients with advanced chronic renal insufficiency have decreased fertility and are therefore much less likely to become pregnant. Renal dysfunction, assessed as, for example, proteinuria and/or decreased GFR, is often exacerbated during pregnancy; however, after delivery, no long-term deleterious effect is seen on

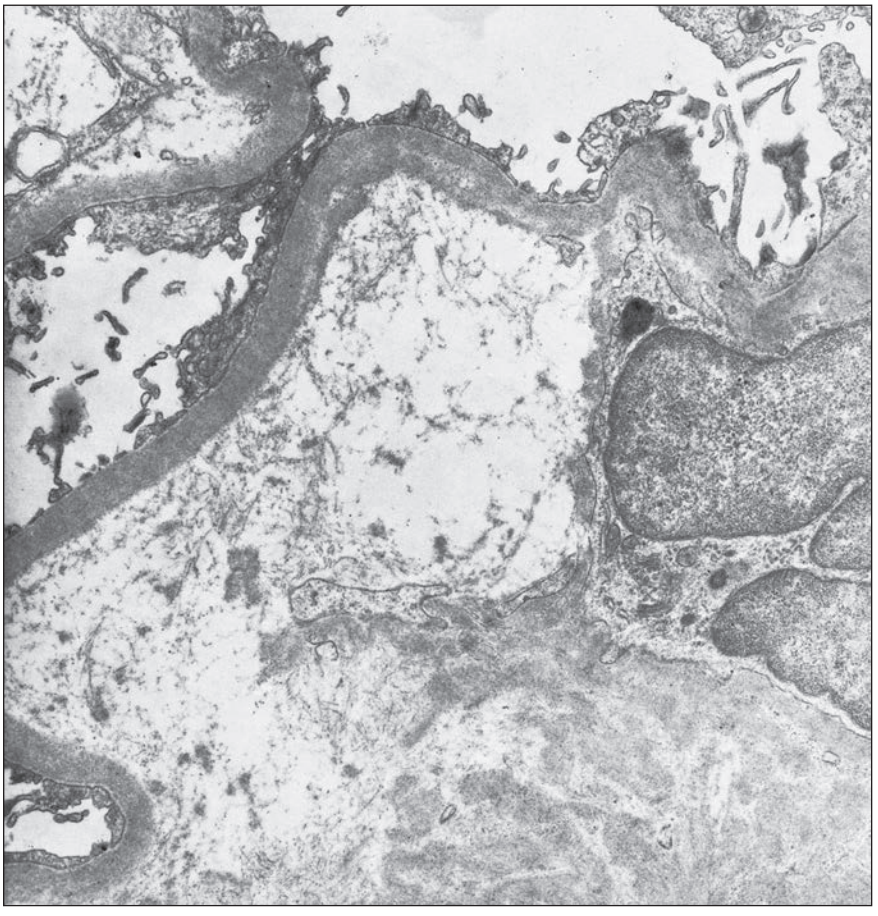


FIGURE 19.18 Lucent expanded subendothelial zone with scattered granular material from a patient with postpartum acute renal failure. (Transmission electron microscopy, ×9000.) (Courtesy of Mary MacDonald, MD.)

TABLE 19.2 Pregnancy and renal diseases: fetal and maternal outcomes

		Effects on the kidney	
Underlying disease	**Effects on pregnancy**	**During pregnancy**	**Long term**
Focal segmental glomerulosclerosis	Fetal loss 20%, prematurity 30%	Decreased renal function in half, especially if abnormal at onset	5% with decreased GFR
IgA nephropathy	Fetal loss 10%, less with normal renal function	Decreased renal function in 24%	No proven effect
Lupus nephritis	Fetal loss 17%, prematurity in ~50%	Persistent or increased activity of active lupus; flare in 35% with prepregnancy quiescence	Poor kidney function with active proliferative lupus; good with mild, quiescent disease
Antiphospholipid antibodies	Fetal loss nearly invariable (~87%)	Thrombosis	Recovery depends on the degree of thrombosis or necrosis
Membranous glomerulopathy	Fetal loss 15%, mostly early, better with less proteinuria	Rare cases of decreased renal function	2% with decreased GFR
Reflux nephropathy	Fetal loss 15%	Hypertension in 20%, UTI in 29%	Decreased GFR in patients with initial renal insufficiency; increased preeclampsia especially with bilateral scars
Diabetes mellitus	Frequent adverse outcome if metabolic control not optimum	Frequent increased proteinuria, hypertension	No proven effect
Granulomatosis with polyangiitis	Unknown	Relapse may occur	Depends on severity of relapse
Antiglomerular basement membrane antibody disease	Unknown	Pregnancy may ameliorate renal disease	Relapse after delivery reported
Polycystic kidney disease	Increased prematurity if complications occur	Increased preeclampsia, especially if hypertensive at onset (54%)	Persistent hypertension as sequela in nearly all who develop preeclampsia
Renal transplantation	Increased fetal toss with renal insufficiency	Increased proteinuria, decreased GFR common	No effect

renal function. Several risk factors for poor maternal and fetal outcomes have been identified. This discussion first focuses on general risk factors for poor long-term renal outcome, then on effects on pregnancy, followed by a brief description of disease-specific aspects of these risks (Table 19.2).

Effects of Pregnancy on the Course of Renal Disease

Transient worsening of hypertension, proteinuria, and renal dysfunction are common during pregnancy in patients with preexisting renal disease. Significant hypertension developed in 23% of pregnancies in one series of women with underlying renal disease. Hypertension occurred more commonly in patients with diffuse glomerulonephritis, focal glomerulonephritis, and arteriolar nephrosclerosis (31). Of these patients who developed moderate to severe hypertension during pregnancy, approximately one half were normotensive before pregnancy. Although genetic risks were not specifically evaluated, the highest elevations of blood pressure occurred in studies with a large proportion of African American patients. Among women with preexisting hypertension in the absence of renal insufficiency, blood pressure elevations were often marked during pregnancy, even rarely leading to abruptio placentae and acute tubular necrosis. Markedly increased proteinuria, nephrotic in 68% of these, occurred in approximately half of the pregnancies, regardless of underlying renal disease (31). In patients with well-maintained GFR and normal blood pressure before conception, there usually were no ill effects of pregnancy on the underlying renal disease. When serum creatinine was 1.4 to 2.5 mg/dL, adverse effects were observed, with increased risk of preeclampsia and preterm delivery. About half of patients with moderate renal insufficiency before pregnancy had a pregnancy-related decrease in renal function, which persisted or worsened after delivery in a fourth (138). If serum creatinine was greater than 2.5 mg/dL, 70% had preterm delivery, 40% had preeclampsia, and 40% had decrease in renal function during pregnancy or after delivery that ultimately lead to dialysis (138).

Restoration of prepregnancy renal function and blood pressure levels occurred in most patients after delivery. However, follow-up at intervals of 3 months to 23 years after pregnancy in this series of 121 pregnancies in 89 women revealed 5 patients with end-stage renal failure and 1 with moderately severe hypertension. The onset of end-stage renal disease was weeks to over 8 years after delivery (31). Risks of permanent deterioration in renal function have been sought. On the one hand, normal renal function at the outset of pregnancy is usually associated with good prognosis for long-term renal function (138). However, in one series of 72 patients with renal disease who became pregnant, 6 of 8 women with a decline in renal function after delivery had normal renal function before

pregnancy (168). Uncontrolled hypertension, nephrotic-range proteinuria, or impaired renal function at the time of conception or at early phases of pregnancy is associated with increased risk of deterioration of renal function (46,138,167,168).

When the diagnosis of renal disease antedates pregnancy, the maternal and fetal outcomes are improved, reflecting intensive medical care by both nephrologists and obstetricians. This point was well illustrated by a large series reported from Melbourne, Australia, which analyzed 395 pregnancies in 238 women with glomerulonephritis (169). Only two patients had renal impairment before pregnancy, and preexisting hypertension was present in 12%. More than half of the pregnancies resulted in hypertension during pregnancy. Hypertension persisted after delivery in 44 patients. Similarly, 59% of pregnancies resulted in increased proteinuria during pregnancy, with persistence in 15% of all patients. Decreased renal function was seen in 15% of pregnancies, with failure to resolve after delivery in 5% of the patients. Eleven women developed irreversible renal dysfunction or worsened renal function with pregnancy. Similar results have been reported in other large series (46,169). Overall, renal dysfunction during pregnancy and outcomes were improved when pregnancy took place *after* diagnosis of renal disease. However, independent of other risk factors, chronic kidney disease still is associated with substantially increased risk of adverse fetal and maternal events (adjusted odds ratio 4.07 for adverse maternal events and 1.76 for fetal events) (170).

Effects of Preexisting Renal Disease on Pregnancy Outcome

Fetal loss and prematurity are increased in pregnancies of women with underlying renal disease when renal function is impaired. In the large patient group from Melbourne described in the previous section, 20% of fetuses were lost and 24% were delivered prematurely (169). Impaired renal function, early or severe hypertension, and nephrotic-range proteinuria were associated with adverse pregnancy outcome (167,169). Renal biopsy lesions of tubulointerstitial injury, arteriolosclerosis, and severe arterial lesions also were associated with unfavorable delivery outcomes (168,169). Additional series show that fetal and maternal outcomes differ in pregnant patients with renal diseases and varying prevalence of associated risk factors. The incidence of normal delivery was highest among patients with membranous glomerulopathy (84%), with 71% and 74% normal deliveries in patients with IgA nephropathy and proliferative glomerulonephritis, respectively (168). Neonatal or fetal death and premature delivery occurred more often when patients had diffuse or focal glomerulonephritis (31). One study demonstrated improved fetal survival in premature infants born to women with renal disease (171). Fetal mortality was only 7% in this group of 67 women with 82 pregnancies, compared with earlier reports with rates of 12% to 88% (167).

Intrauterine growth restriction is an additional important adverse event with profound consequences for the adult health of the offspring. Low birth weight term babies have increased cardiovascular and renal disease risk in adulthood (172,173). The mechanism has not been proven, but low birth weight has been proposed to be linked to development of fewer nephrons, with resulting increased risk for hypertension and progressive renal disease in adulthood (173). Whether incidence of low birth weight of term births is increased in pregnancies of patients with renal disease has not been proven. Experimental evidence supports the concept that the induction of low birth weight by introducing intrauterine ischemia in pregnant rats could result in low birth weight offspring with increased hypertension in adulthood (174). In contrast to these studies in the rat, in a sheep model with intrauterine growth restriction owing to late gestational umbilicoplacental embolization or natural twinning, there was no decrease in nephron number with the late induction of intrauterine growth restriction, whereas growth restriction owing to twinning did result in decreased nephron number. These results suggest that not only is growth restriction of importance in determining nephron endowment but the timing of the insult that decreases growth is crucial for effects on nephron development (175). Both maternal and genetic factors have been proposed to influence nephron endowment, including, for instance, maternal nutrition, altered hormones, or toxins or genetic factors (176). Thus, severe dietary protein restriction in midgestation, as seen in the Dutch famine of 1944/1945, was associated with increased adult hypertension and microalbuminuria (177). Animal studies support the concept that the mechanism of malnutrition on nephron number could be through impairment of renal development (178,179).

Pathogenesis

Complex changes occur in renal function during normal pregnancy (see p. 816), some of which are postulated to have adverse effects on renal disease. However, in contrast to the loss of nephrons, glomerular hyperfiltration and hypertension, and increased growth factors, which occur in many progressive renal diseases, pregnancy is a physiologic state of moderate renal vasodilatation without increased glomerular pressure or growth (1). Because direct assessment of many of these parameters is not possible in humans, animal models have been studied to determine the effect of pregnancy on the kidney and on preexisting renal disease. As in humans, mild azotemia induced transiently by uninephrectomy did not have an adverse effect on pregnancy outcome. Although rat pups were born with glomeruli with larger volume, no long-term effect on glomerular growth or development was detected by 6 weeks after birth (180). Repeated normal pregnancy also showed no adverse effects on the kidney. In rats with hyperfiltration maintained by repeated pregnancies and lactation for 6 months, no adverse effects were seen on subsequent renal function, even with additional stimuli that increase hemodynamic load, such as high-protein diet and uninephrectomy (1). Glomerular pressures did not increase in these experimental settings despite the vasodilation of pregnancy, because of equal decrease in resistances of afferent and efferent arterioles (1).

When pregnancy was superimposed on experimental rat models of human membranous glomerulopathy, antiglomerular basement membrane disease, or focal segmental glomerulosclerosis, no additional deleterious effects on pregnancy, renal function, or sclerosis resulted (181–185). Even repeated pregnancies did not worsen sclerosis in the remnant kidney model of focal segmental glomerulosclerosis (186). Micropuncture studies showed decreased glomerular pressures with pregnancy in the remnant kidney model (183). Experimental membranous glomerulopathy also led to decreased glomerular pressures in pregnant compared with nonpregnant rats, as a result of systemic decrease in blood pressure and glomerular vasoconstriction at both the afferent and efferent arterioles (181). These models do not mirror the human situation, in which pregnancy occasionally can lead to rapid and irreversible worsening of underlying renal

disease. The next studies are of particular interest in this regard. In contrast to the foregoing models, rats with mild doxorubicin (Adriamycin®) nephropathy, another model of focal segmental glomerulosclerosis, developed renal and systemic vasoconstriction, increased blood pressure, and proteinuria in response to pregnancy (185). These preeclamptic-like responses were partially corrected by L-arginine, which stimulates nitric oxide release. In normal rats, nitric oxide synthesis appears increased during pregnancy (186). However, endogenous synthesis of nitric oxide could be inadequate when renal disease is superimposed on pregnancy, perhaps because of underlying endothelial dysfunction. Additional key vasoactive compounds, such as VEGF or PAI-1 (see Preeclampsia Discussion pp. 833–834), which normally increase in pregnancy, could also be altered when there is underlying chronic kidney disease, with resulting endothelial and fibrotic/thrombotic injuries. Experimental evidence supports the hypothesis that decreased VEGF and increased PAI-1 are linked to accelerated kidney fibrosis (187,188).

Specific Renal Diseases and Pregnancy

Focal Segmental Glomerulosclerosis

In several series, focal segmental glomerulosclerosis was the underlying renal disease associated with the highest incidence of fetal and maternal complications (169,189,190). Impairment of renal function occurred in nearly 50% of pregnancies in this diagnostic group compared with only 5% among patients with non-IgA diffuse mesangial proliferative glomerulonephritis. Decreased renal function occurred in 15 of 31 pregnancies in 21 women with primary focal segmental glomerulosclerosis. In four patients, renal dysfunction was irreversible, and three of these patients progressed to end-stage renal disease. This series included patients who had focal and segmental hyalinosis and sclerosis, which may represent a group with worse prognosis and more advanced disease (191,192).

Lupus Nephritis

The risk of exacerbation of lupus nephritis with pregnancy varies widely, from 8% to 74% (193). A prospective study suggests that pregnancy per se increases risk of exacerbation of lupus nephritis (138). In patients with mild systemic lupus erythematosus (SLE), pregnancy does not appear to affect the course of lupus, although pregnancy complications may be increased (193). Poor outcome is seen in patients with active proliferative lupus nephritis (World Health Organization or International Society of Nephrology/Renal Pathology Society class IV) before conception. In a comprehensive review by Hayslett (194), patients with disease activity at conception showed persistent or increased activity of SLE in 52%, compared with sustained remission in 65% of patients with quiescent SLE before conception. In a prospective series of patients with lupus nephritis from France (195), SLE exacerbation during pregnancy was increased in patients with a past history of fetal loss, proteinuria, or active SLE at the onset of pregnancy, hypertension, or absence of anti-SSA antibodies. Relapses of SLE during pregnancy occurred in 27 of 75 patients with inactive SLE before conception, and 7 additional patients had a relapse postpartum. Nearly half of the births were premature, and fetal loss occurred in 18 of 103 pregnancies (195). In a series reported by Packham et al. (196), no progression to end-stage renal failure and no maternal deaths were seen, in contrast to a 3.4% death rate reported in a review by Cameron and Hicks (197). This lower rate of exacerbation reported by Packham et al. appears to reflect that most patients in this series became pregnant only after remission of their disease. An Italian study of 70 pregnancies in 48 women with lupus nephritis showed development of SLE during pregnancy in 13 patients. Pregnancy loss was similar to those whose diagnosis of SLE preceded pregnancy (38% vs. 35%) and was associated with hypertension, proteinuria, and antiphospholipid antibodies. Prematurity was common, occurring in 13 of 41 live births. Renal flares were common in those with preexisting SLE, developing during pregnancy in 12 of 38 patients. Favorable outcome was linked to quiescence of renal disease at onset of pregnancy (198).

Results from a single center described increased complications during pregnancy in SLE patients with lupus nephritis versus those without lupus nephritis or with quiescent lupus nephritis (199). In a recent meta-analysis encompassing 2751 pregnancies in 1842 women, lupus flare was present in 25.6%, hypertension in 16.3%, preeclampsia in 7.6%, and eclampsia in 0.8%. Spontaneous abortions occurred in 16%, stillbirths in 3.6%, with neonatal deaths in 2.5%, and intrauterine growth retardation in 12.7% (192). Both lupus nephritis and antiphospholipid antibody were linked to hypertension and premature births. Deaths in the postpartum period in patients with lupus nephritis were all linked to active disease and attributed to infection or disease activity (200).

Differentiation of flares of lupus nephritis from preeclampsia may be difficult, and it is also critically important therapeutically, because high-dose prednisone is commonly used for SLE and may be contraindicated in preeclampsia (201). Previous studies have suggested the use of low C4 levels to distinguish SLE exacerbation from preeclampsia (202); however, low C3 or C4 levels may occur in preeclampsia (203).

Antiphospholipid Antibodies

Antibodies to phospholipids increase in prevalence in patients with SLE during pregnancy and are associated with poor outcome. Antiphospholipid antibodies play an important role in the pathogenesis of vascular lesions in patients with SLE, and high antibody titers correlate with vascular thrombosis, decreased renal function, hemolytic anemia, thrombocytopenia, and fetal loss and may promote decidual vasculopathy and placental insufficiency (198,204). In one series (205), only 2 of 23 pregnancies in 12 women with these antibodies were successful. Patients also have increased risk for chronic decreased renal function.

These antibodies may occur in patients with SLE also as part of the antiphospholipid syndrome. Patients with this syndrome have recurrent arterial or venous thrombosis, livedo reticularis, and recurrent fetal loss. Preeclampsia has also been linked to these antibodies. The prevalence of antiphospholipid antibodies among women with preeclampsia has been reported to be as high as 20% in some series (206–209). In a prospective study, patients with lupus nephritis were assessed for development of antiphospholipid antibodies. These antibodies were associated with increased development of chronic kidney disease and with fetal loss (210). Postpartum renal disease has been reported in 12 cases with antiphospholipid antibodies, 8 after fetal death and 5 after preeclampsia (157,211).

Biopsy specimens taken during or soon after pregnancy in seven patients with antiphospholipid antibodies, one of whom also had lupus nephritis, showed acute fibrinoid lesions with fibrin thrombi in glomeruli, arterioles, and arteries. Glomeruli

showed double contours of capillary walls, which persisted in later follow-up biopsies performed in five of these patients. Chronic vascular injury was also present in these late biopsies, with narrowing of arteries resulting from recanalizing thrombi and cellular intimal proliferation (205). Vasculitis involving medium-sized and smaller arteries with marked infiltration of the vascular wall by lymphocytes, neutrophils, and occasional eosinophils with focal fibrinoid necrosis was associated with antiphospholipid antibodies in a pregnancy with prolonged intrauterine fetal demise (211,212). Placental vascular lesions in one case of a patient with lupus anticoagulant were similar to those described in preeclampsia (213). Electron microscopic studies at the acute stage showed translucent glomerular subendothelial deposits and fibrin, typical of the acute phase of thrombotic microangiopathy. In biopsies performed 1 year or more after pregnancy, persistent double contours of glomerular capillaries with mesangial interposition were present.

Therapy with low-dose aspirin and possible additional heparin has been advocated for patients with antiphospholipid antibodies in pregnancy (214).

IgA Nephropathy

IgA nephropathy and other mesangial diseases are associated with a moderately good prognosis for pregnancy outcome. In one large series of patients with pregnancy and various glomerulonephritides, mesangial (non-IgA) glomerulonephritis was associated with the best prognosis (169). The incidence of normal delivery was 71% in patients with IgA nephropathy in one large series from Japan (168). Fetal loss or neonatal death occurred in 10% of pregnancies, and spontaneous abortion occurred in 3%.

A prospective study of IgA nephropathy patients with stage 2 (GFR 60 to 89 mL/min; normal greater than 90 mL/min) or stage 3 (GFR 30 to 59 mL/min) chronic kidney disease in Japan showed that pregnancy was not a risk factor for increased progression, although there was transient increase in proteinuria during pregnancy (215). Similarly, a study from Italy found that pregnancy in IgA nephropathy patients did not affect long-term GFR changes, proteinuria, or development of new-onset hypertension (216). Chronic deterioration of renal function in IgA nephropathy rarely can be linked to gestation. Reversible decline in renal function is much more common, occurring in 24% (217). In a prospective study of 71 women with biopsy-proven IgA nephropathy, no difference in pregnancy outcome or renal function was seen between those who conceived and those who did not (218). Four of the pregnant patients and two of the nonpregnant patients reached end-stage renal disease during the 5-year follow-up. Entry biopsies in these patients already showed moderate to advanced diffuse proliferative glomerulonephritis with advanced tubular atrophy, interstitial fibrosis, and arteriosclerosis. Superimposed focal and segmental hyalinosis and sclerosis or diffuse mesangial proliferation were indicators of poor prognosis in another large series. Approximately one third of patients with these biopsy changes showed renal dysfunction, increased proteinuria, or increased blood pressure during pregnancy (169). The highest incidence of maternal complications was correlated with those biopsies with superimposed focal and segmental proliferative lesions (219). The presence of active crescents or more than 10% sclerosed glomeruli was not associated with statistical differences in fetal or maternal outcome. Because these features have been associated with poor outcome in nonpregnant patients, it is possible that the relatively small number of patients with these lesions prevented detection of a specific effect.

Membranous Glomerulopathy

Membranous glomerulopathy in general is associated with a favorable renal prognosis and an ability to carry pregnancy to term (197). Only 3 cases of irreversible decline in glomerular function were reported in series totaling 132 patients (220). Thirty of the 193 pregnancies (15.5%) in these women resulted in fetal loss. Nephrotic-range proteinuria during early pregnancy was associated with poor fetal outcome and irreversible hypertension or renal impairment. No correlation was found between stage of membranous glomerulopathy (see Chapter 7) and outcome in this group.

Reflux Nephropathy

Combining published series of reflux nephropathy from Kincaid-Smith and Fairley (221), Jungers et al. (222), and Arze et al. (223) gives results from 772 pregnancies in 320 women. Overall, fetal deaths occurred in 15% of pregnancies, hypertension in 20%, and UTI in 29%. When only persistent vesicoureteric reflux was present, there was no increased fetal or maternal risk (16). Moderate renal insufficiency at the onset of pregnancy in a large group of women with reflux nephropathy was associated with augmented hypertension and increased decline in renal function (191). Patients with reflux who have increased serum creatinine are at increased risk for preeclampsia (222). This incidence is particularly high, 24%, in women with bilateral scars, compared with only 7% in patients with only unilateral scars (16,191). In a prospective study, preeclampsia developed in 24% of 46 women with 54 pregnancies, most often in those with preexisting hypertension. Decreased renal function during pregnancy occurred in 18%, and preterm delivery occurred in a third (138).

Systemic and Genetic Diseases

Diabetes Mellitus

Prematurity and complications in neonates are well known to occur in diabetic patients, and they are particularly common in patients with impaired renal function during the first trimester. Diabetic patients without nephropathy have fewer fetal complications. Increased renal dysfunction during pregnancy, characterized by increased proteinuria and serum creatinine, is common in patients with diabetic nephropathy. More serious complications may also occur. In a study of 40 pregnancies in 33 women with diabetic nephropathy, 7 developed a preeclampsia-like syndrome, and decline in renal function was significantly greater in those patients with elevated serum creatinine at the beginning of pregnancy. However, the average rate of fall in creatinine clearance (0.65 mL/min/mo) in diabetic patients is only slightly higher than the range observed prospectively in studies of diabetic nephropathy treated with antihypertensive agents (224). A prospective study examined the effect of stage of diabetic kidney injury before pregnancy on outcomes. Preterm delivery was observed in 91% of type 1 diabetic women with overt diabetic nephropathy versus only 35% of those without albuminuria. Preeclampsia developed in 64% of those with overt diabetic nephropathy, 42% of those with microalbuminuria, and only 6% of those without albuminuria. Renal dysfunction deteriorated in those with overt diabetic nephropathy before conception. In contrast, renal

disease did not progress in patients with normal GFR and blood pressure before pregnancy (138).

Autosomal Dominant Polycystic Kidney Disease

Preeclampsia occurs in increased frequency (9%) in pregnancies in patients with autosomal dominant polycystic kidney disease. When superimposed preeclampsia occurred, prematurity rates were significantly increased (138,225). The incidence was particularly high in patients who were hypertensive at the onset of pregnancy versus those who were normotensive (54% vs. 8%). Of the 26 women who developed preeclampsia or eclampsia, 23 subsequently developed persistent hypertension. The risk for UTI is also increased in pregnancy in these patients (138).

Granulomatosis with Polyangiitis and Microscopic Polyangiitis

Several reports suggest the possibility that pregnancy could have an adverse effect on granulomatosis with polyangiitis, previously known as Wegener granulomatosis (226,227). Relapse of kidney involvement occurred in five of eight pregnancies in women with known granulomatosis with polyangiitis (226). Fifteen pregnancies in 10 women with granulomatosis with polyangiitis were reviewed in one series, with diagnoses made during pregnancy or postpartum in 7 of these cases (226). Risk factors for relapse in pregnancy were not identified. One patient completed two normal pregnancies without relapse before a mild relapse occurred in a third pregnancy. Relapse did not occur in a patient with kidney transplant who was maintained on immunosuppression. Relapse affected the liver in one patient with stable renal function before pregnancy, and it resulted in fibrinoid necrosis of hepatic parenchyma and fetal loss (228). In a more recent survey of the literature, 28 pregnancies in patients with granulomatosis with polyangiitis were identified (229). The diagnosis of granulomatosis with polyangiitis was made during pregnancy in eight. Nineteen of 27 cases with outcomes recorded resulted in live births, 7 pregnancies terminated in abortions, and 2 maternal deaths occurred. Microscopic polyangiitis has only very rarely been reported in pregnancy (230), perhaps reflecting its lesser propensity to relapse than granulomatosis with polyangiitis. A single case demonstrated the transmission of myeloperoxidase-specific antineutrophil cytoplasmic antibodies (MPO-ANCA) across the placenta from a mother with microscopic polyangiitis, resulting in pulmonary hemorrhage and renal abnormalities developing shortly after delivery in the baby. The cord blood showed the presence of the same MPO-ANCA as in the mother's serum. The baby showed an excellent clinical response to exchange transfusion and immunosuppression, and the mother also responded to therapy (231).

Anti–Glomerular Basement Membrane Antibody Disease

In contrast to the experience in most kidney diseases, several case reports suggest that pregnancy may temporarily ameliorate activity of anti–glomerular basement membrane antibody disease. Detailed studies were performed in a patient who developed renal disease during pregnancy, with marked deterioration of renal function after delivery at 35 weeks' gestation. A renal biopsy performed 11 days postpartum revealed crescentic glomerulonephritis with anti–glomerular basement membrane antibodies. Studies of the patient's serum documented that her anti–glomerular basement membrane antibodies could bind to placental membranes. The infant showed no evidence of renal disease, and the infant's serum did not contain anti–glomerular basement membrane antibodies. The rapid decline of renal function postpartum was postulated to have been due in part to removal of the ameliorating influence of the placenta (232). An additional case of rapid decline in renal function postpartum has been described, although further studies were not performed in this case (233).

Other Systemic Diseases

In reviews of effects of pregnancy on scleroderma, no significant effect of pregnancy on kidney disease could be established, although there is increased premature or small for age babies (234,235). Although individual case reports have described renal crisis during pregnancy, this condition does not appear to occur at an increased frequency during pregnancy (235,236). In amyloidosis, as in other renal diseases, more severely compromised renal function at conception was associated with deterioration of renal function during pregnancy (237).

Kidney Transplant

The effect of pregnancy on renal function in renal allografts has been studied in detail. Recent studies have not found the apparent adverse effect observed in older series (238–240). No adverse effects of pregnancy on graft function were detected in a series of 113 pregnancies in 73 transplanted women (241). Premature delivery occurred in 64% of the pregnancies, with no congenital defects or renal functional defects, hypertension, or proteinuria observed in these babies, followed on average till age 52 months (242). In several large series, comparing matched male or nonpregnant female cohorts with transplant recipients who became pregnant, no adverse long-term effect on renal allograft function or survival was detected (238). Although creatinine clearance decreased late in pregnancy in renal transplant patients to a greater extent than in healthy women, permanent impairment of renal function was not typical. Proteinuria was also increased slightly during pregnancy, to approximately 200 mg/24 h versus 150 mg/24 h in normal subjects at comparable time of pregnancy. By the third trimester, proteinuria in renal transplant patients was three times that of nonpregnant levels, returning to prepregnancy levels by 2 to 3 months after delivery (1). In an additional case-control study, no significant difference was found in plasma creatinine levels after 15 years of follow-up (238).

Patients with decreased renal function who also are receiving immunosuppression have decreased fertility. When renal transplant patients do conceive, spontaneous abortions are increased if significant renal insufficiency is present, whereas a good pregnancy outcome is associated with intact renal function (243).

Renal Cancer

The apparent increase in the number of cases of renal cancer in pregnancy may reflect increased incidental detection during pregnancy because of the routine use of ultrasound. Forty-four cases of renal cell carcinoma discovered during pregnancy were reported in one review (244). Formerly, palpable flank masses were the most common presentation, in contrast to early detection of smaller lesions with the use of high-resolution ultrasound. Presenting symptoms, when present, are usually suggestive of recurrent UTI (244). Parity was associated with increased risk for renal cancer in several cohort studies, but mechanism(s) and potential causality remain unclear (245).

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CHAPTER 20

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Renal Disease Caused by Hypertension

DEFINITION

Hypertension, or increased blood pressure, is a worldwide problem with a prevalence of greater than 1 billion people (1). The Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7) has published guidelines for the classification of different levels of blood pressure (1) as follows: normal pressure is considered to be less than 120/80 mm Hg; prehypertension is 120 to 139/80 to 89 mm Hg; stage 1 hypertension is 140 to 159/90 to 99 mm Hg; and stage 2 hypertension is greater than 160/100 mm Hg. The percentage of Americans with hypertension has remained at approximately 29% since 2000 according to the 2007–2008 National Health and Nutrition Examination Survey (NHANES) data (2). Control of hypertension has improved from 27.3% in 1988–1994 to 50.1% in 2007–2008 due to increased awareness, treatment of more of the aware patients, and control in more of those who were treated (2).

Hypertension can have a known cause, such as renal artery stenosis, but in most cases, the etiologic factors are unknown, and the condition is deemed "essential" hypertension. The prevalence of the secondary forms of hypertension, in which the cause can be determined, is generally thought to be less than 10% (3). This chapter covers all forms of hypertension but focuses mainly on essential hypertension.

ESSENTIAL HYPERTENSION

Clinical Presentation

Prevalence, Gender, and Age

The global prevalence of hypertension was estimated at 26% in 2005 and is expected to increase to 29% by 2025 (4). The prevalence of hypertension in the United States as determined by an analysis of data from the NHANES was found to be 29% (5). The age-standardized prevalence rate showed an increase from 24% in 2000 (6). Much of this increase can be explained by the increase in overweight/obesity in the population (6). Other factors include improved survival due to effective treatment, increased sodium intake, and a more sedentary lifestyle (6). Men have slightly higher prevalence of hypertension than women. However, in the interval between 2000 and 2005, the prevalence increased in women but not in men (6). Hypertension is a well-known risk factor for chronic kidney disease (CKD) and is currently the second most common diagnosis for patients with prevalent end-stage renal disease (ESRD) (7). However, the percentage of patients with primary hypertensive nephropathy is difficult to determine as such patients are not usually biopsied, and this diagnosis is often made by exclusion.

Although essential hypertension first becomes manifest in middle life, evidence suggests that factors such as low birth weight due to maternal malnutrition may contribute to its development later in life (8–10). Small placental size correlates to higher blood pressure even in early childhood (9). Others have related low birth weight and diminished nephron numbers to a higher risk of hypertension in adolescence or adulthood (8,10). The prevalence of hypertension in children has risen in the last decade from a range of 1%–2% in the 1970s to 3%–5% in the early 2000s (11). This increase has occurred

chiefly in essential hypertension and is explained in large part by an increase in the body mass index (BMI) or waist circumference (11,12). Other risk factors include low birth weight, inflammation, elevated uric acid, and high dietary salt intake (12). Childhood hypertension and prehypertension predict increased blood pressure as an adult. Systolic blood pressure rises throughout life with a difference of 20 to 30 mm Hg from early to late adulthood (13). More than half of all individuals aged 60 to 69 are hypertensive, and this proportion increases to 75% of those aged 70 years and older (1). Diastolic blood pressure rises but to a lesser degree and only until the sixth decade (1). In general, cardiovascular risk is related to levels of diastolic pressure in patients under age 60. However, pulse pressure (the difference between systolic and diastolic pressure) is the best predictor of cardiovascular risk in patients older than 60 years as it is a marker of central vascular stiffness (14). Secondary forms of hypertension should be considered in patients less than 30 years of age and in those older than 60 years with sudden onset of hypertension. A careful family history should also be obtained, because many of these forms have a genetic basis.

Genetic Factors

Essential hypertension is a polygenic quantitative trait with only a small contribution from each gene and with modulation by other genes (epistasis), as well as factors such as gender, race, age, and environment. Epigenetic mechanisms that alter gene expression without changing the DNA sequence of the gene are also at play (15). The genetic contribution to control of blood pressure has been estimated at 31% to 68% (16). The evidence of a genetic effect was first recognized by family and twin studies. Newer approaches include discovery of individual candidate genes, genome-wide linkage studies (GWLS), and genome-wide association studies (GWAS). However, less than 3% of the observed variance can be attributed to the genetic variations found to date (16). Additional information has been provided by the examination of experimental animal models of hypertension (17).

Family and twin studies provided the first evidence of genetic factors in essential hypertension. In one such study, Mo et al. (18) studied 520 offspring with two hypertensive parents, two normotensive parents, or one of each. Those offspring who had two normotensive parents had a mean blood pressure of 121 mm Hg, and 1.3% of them were taking antihypertensive drugs. The intermediate group with one parent of each type showed an average blood pressure of 125 mm Hg, and 2.4% of them used antihypertensive drugs. The last group with two hypertensive parents had an average blood pressure of 135 mm Hg, and 11.7% had been prescribed antihypertensive medications. These differences were significant among all three groups. Twin studies are even more powerful because it is likely that any differences are due to environmental influences in the case of monozygotic twins but that of dizygotic twins represent both genetic and environmental differences. Cui et al. (19) found a univariate correlation coefficient of systolic blood pressure of 0.78 between monozygotic twins, 0.50 between dizygotic twins, and 0.12 between spouses.

Investigators at the end of the 20th century expected that they would be able to discover several candidate genes that might be responsible for essential hypertension (20). Candidate gene studies are hypothesis driven as the genes are chosen on the basis of our knowledge of the intersection of pathophysiology of hypertension and potential molecular pathways. Such studies have been successful in finding more than 15 genes responsible for monogenic heritable forms of hypertension (16,21). Many of these will be discussed below in the section on secondary forms of hypertension. The first demonstration of a significant polymorphism in a candidate gene in essential hypertension was in the study by Jeunemaitre et al. (22) of the angiotensinogen (AGT) gene. They found that hypertensive members of sib-pairs in two large populations inherited a variant of AGT with threonine rather than methionine at position 235 (235T) in higher frequency than normotensive members of the pair. Furthermore, these hypertensive patients had higher concentration of AGT in their sera. They concluded that this polymorphism might contribute to the hypertension. The next step was to determine how the polymorphism affects the function of the protein. Hopkins et al. (23) found that homozygous patients with the 235T genotype had a blunted renal vascular response to the infusion of angiotensin II (AII). Studies of candidate genes in essential hypertension have identified over 50 genes in pathways thought to be involved in essential hypertension including the renin-angiotensin system (RAS), renal sodium and water handling, ion channels and cotransporters, kinase regulators, and enzymes and receptors involved in aldosterone synthesis and signaling pathways (Table 20.1) (16,24). However, such studies are limited as they ignore epistasis, gene-environment interactions, and rare variants. These limitations sometimes lead to conflicting results.

The next development in the determination of the role of specific genes in hypertension was the use of genome-wide linkage scans (GWLS), which allowed the discovery of unsuspected genes and pathways as well as rare variants that had large effects on blood pressure. Most of these GWLS reported associations only, and many were not confirmed (16). A few of these studies were successful with the results confirmed by others. For example, such analysis by Chang et al. (48) found a region on chromosome 1q that contained at least three genes that associated with blood pressure levels. The three genes included *ATB1B, RGS5*, and *SELE. ATB1B* encodes the β subunit of the sodium/potassium ATPase, which is involved in sodium

TABLE 20.1 Candidate genes for essential hypertension

Adducin (25,26)
Adrenergic receptors (27,28)
Aldosterone synthase (29,30)
Angiotensin converting enzyme (31–33)
Angiotensinogen (29,31)
Angiotensin II type 1 and 2 receptors (29,34)
Apolipoprotein E (35)
Bradykinin receptors (36)
Dopamine β-hydroxylase (37)
Endothelial NO synthase (28,38)
Endothelin (39,40)
Epithelial sodium channel (41,42)
Renin (27)
Sodium-calcium exchanger (43)
Transforming growth factor–β (44,45)
With-no-lysine kinase 1 (46,47)

These and additional candidate genes are also listed in Table 2 in Basson et al. (16).

absorption in the kidney, heart contraction, and regulation of vascular smooth muscle tone. *RGS5* inactivates the G proteins involved in vasoconstriction that is mediated by angiotensin. It may also invoke vascular remodeling and angiogenesis. *SELE* affects vasoactive response. Better detection of possible genes can be achieved by the incorporation of age-genetic interactions into the GWLS (49). This method also leads to improved validation. The results of GWLS can be further improved by the use of meta-analysis (50).

GWAS were next undertaken in order to find common variants with small effects that are missed in the candidate gene or linkage analyses. Such studies when combined with meta-analysis and huge sample size have defined more than 50 candidate genes that account for less than 3% of blood pressure variability (16). The first two large GWAS meta-analyses were performed in European populations and uncovered 13 new loci for blood pressure traits such as systolic and diastolic blood pressure (51,52). A more recent study by Ehret et al. (53) discovered 16 novel loci also using a European population. Many of these loci were also associated with determination of blood pressure in people of East Asian, South Asian, or African descent (53). Similar methodology studying such traits as pulse pressure and mean arterial pressure uncovered six additional loci (54). Many candidate genes within the loci in all of these studies are reasonable candidates to affect blood pressure regulation or to influence blood pressure directly. Many of the original single nucleotide polymorphisms (SNP) found in these studies have been replicated in later studies (16), indicating the value of such studies.

As the complexity of genetic control of blood pressure regulation has become apparent, the effects of epigenetic mechanisms and epistasis have been revealed. Epigenetics refers to mechanisms whereby environment-gene interactions can occur via DNA methylation or changes to histones that do not alter the DNA sequence yet change DNA expression. These epigenetic modifications occur in response to environmental stressors such as maternal malnutrition and may remain in effect for several generations. Several such epigenetic mechanisms are reviewed by Millis et al. (15). Epistasis, the modulation of the expression of one gene by another, is also being increasingly recognized and complicates the interpretation of candidate gene studies (16,55).

Systems biology including the study of pathways is an emerging area of research that is likely to be critical to our understanding of the genetic control of blood pressure (16,56). The usefulness of this approach is illustrated by an examination of the fibroblast growth factor (FGF) signaling pathway and its relationship to hypertension (57). A GWAS had identified that a particular SNP of *FGF1* was associated with essential hypertension in a large Polish kindred and that its product was increased in the kidney in patients with essential hypertension (58) even though this was not a pathway that had originally been thought to be important in blood pressure regulation. These investigators then explored genes in the FGF signaling pathway and found additional allelic variants of genes, namely FGF-binding protein, that also segregated with essential hypertension (57) and were up-regulated in the kidneys of hypertensive patients as compared to controls.

Experimental models of hypertension chiefly in mice and rats have been used to further dissect the genetics of hypertension (55). It is beyond the scope of this chapter to review all of this work, but a few representative examples are given. Cvetkovic and Sigmund reviewed the early use of knockout and transgenic mice (59). Graham et al. (60) applied genome-wide screening to the stroke-prone SHR and then applied congenic/consomic breeding models to confirm the quantitative trait loci and dissect the genes further. The role of experimental models in defining new candidate genes is illustrated by renalase, an amine oxidase that lowers blood pressure (61). Renalase knockout mice are hypertensive as are rats with a small inhibitory mRNA. These experiments prompted a search for SNPs in the renalase gene in human beings. Two such SNPs were found that were associated with essential hypertension (61). Various experimental models of hypertension, genetic, and otherwise have been reviewed by Dornas and Silva (17).

Genetic background may also be used in the future to determine the best therapy for individuals (55). For example, Matayoshi et al. (62) examined polymorphisms in 17 genes and found 2 (one each in the thiazide-sensitive cotransporter gene and adrenergic receptor-beta 3 gene) that were associated with a good therapeutic response to thiazide diuretics that was not seen in those patients without the polymorphisms. Other investigators have begun to study other polymorphisms with an eye to tailoring therapy to the individual patient. This is described further in the section on "Therapy" (see below).

Thus, it is evident that hypertension does not rely on a single gene but is a polygenic complex trait as first predicted by Hamilton et al. (63) in 1954. Although a few single genes may cause hypertension by themselves, most genetic effects act by increasing susceptibility to develop hypertension under certain conditions. Future studies will be aimed at additional gene discovery, dissection of the pathways that lead to regulation of blood pressure and parsing the relationships among the genes and their regulation whether by modulation by other genes, by epigenetic mechanisms, or by interactions with the environment.

Environmental Factors

Several studies have shown clearly that environmental factors are important in the control of blood pressure (1,64,65). Individual factors that have been examined include low birth weight, BMI, physical activity, dietary factors including salt intake, smoking, alcohol consumption, cocaine abuse, use of oral contraceptives (OCs), and psychological stress. The effects of many of these risk factors depend on an interaction between genes and the environment. This interaction is illustrated by the case of intrauterine growth retardation. Decreased nutrition to the fetus can result in the epigenetic changes of altered methylation of DNA or histone acetylation that then may result in changes in gene expression causing variation in the numbers of stem cells committed to different lineages (8,64). The decrease in nephron numbers can lead to hypertension in adult life. Lifestyle risk factors may be beneficial or harmful. In an individual, blood pressure decreases in a linear fashion with weight loss (66). Physical activity or fitness independent of weight loss also reduces blood pressure (66). Zhao et al. (65) reviewed the literature for the effect of 27 dietary factors. They found that 19 were protective and 6 were associated with an increased risk for hypertension. The strongest protective factors were a diet high in fruits and vegetables, the Dietary Approaches to Stop Hypertension (DASH) diet, and to a lesser extent, increased potassium, calcium, magnesium, and polyunsaturated fatty acid intake. The major dietary risk factor was increased sodium intake.

The evidence for this risk is overwhelming. Numerous studies (reviewed by He and MacGregor (67)) have confirmed the risk and have demonstrated that reduction in dietary salt reduces blood pressure. Smoking, alcohol consumption, and cocaine abuse are all known to increase blood pressure acutely (1), but chronic effects are less clear. OCs are also associated with high blood pressure, and this risk is even greater with advancing age, duration of use, and increased body mass (1). Stress may increase blood pressure acutely but does not cause chronic hypertension (68). However, a review of several studies suggestive of this association supports the possibility that a chronic nonadaptive response to stress may result in hypertension (68).

Racial Factors

The prevalence of hypertension in non-Hispanic black men and women is 40.1% compared to 27.4% for non-Hispanic whites (6). The prevalence of hypertension among Mexican Americans is 27.1%. These data are derived from the 1999–2004 NHANES (6). Hypertension begins at an earlier age, is more severe, and is more difficult to control in African Americans (69,70). Blacks also progress to ESRD owing to hypertension 17.7 times more frequently than do whites (71). Parmer et al. (71) examined the effects of high- and low-salt diets on a group of white and black men matched for blood pressure and found an impaired ability to excrete a salt load in the black group. Proposed causes for this altered salt handling in blacks include failure to respond appropriately to AII, reduction in kallikrein-kinin excretion, and hyperinsulinemia (72). As noted earlier, low nephron number has also been associated with the development of hypertension, and blacks as a group do have lower birth weights and likely lower nephron number (10,73). Differences in the pathology of hypertensive-associated renal disease between African Americans and whites have been described (74). Moreover, recent evidence originally suggested that the apparent increase in hypertensive renal disease in African Americans may be secondary to the high frequency of mutations in the nonmuscle myosin heavy chain 9 (*MYH9*) gene found in this population (69). The strong association between hypertensive African Americans with renal disease and polymorphisms of the *MYH9* gene supported the possibility that the renal disease was primary rather than representing hypertensive nephropathy (75). More recently, it has been found that the risk for various forms of CKD in this population is more strongly related to missense mutations in apolipoprotein 1 (APOL1), which is closely linked to MYH9 (76). The functions of APOL1 include among others association with circulating HDL and autophagic pathways; both functions could play a role in hypertensive injury or other forms of kidney disease (76).

Clinical Features

Initial Presentation

In the earliest stages, most patients are asymptomatic, and the condition is recognized only at routine examination, when injury to the vessels may already be present. Symptoms that may be recognized include headache, epistaxis, tinnitus, and dizziness, but symptoms are unusual. Excessive weight, smoking, abnormal serum lipid patterns, and diabetes mellitus are common. Repeated blood pressure readings are necessary to establish the diagnosis and to determine the appropriate therapy (77). The prevalence of hypertension is increasing in children. As in adults, these patients do not present with symptoms but are detected on routine evaluation. Hypertension in children is defined as the average of at least three determinations of systolic and/or diastolic blood pressure ≥95% for gender, age, and height (78). Kaelber and Pickett (79) have devised a table to help determine which children should undergo further evaluation following a first reading of elevated blood pressure.

As the duration of hypertension lengthens at any age, evidence for complications may become manifest. The target organs of hypertension include the heart, brain, peripheral arteries, eye, and kidney (80,81). Hypertension accelerates atherosclerosis in the systemic circulation, whereas the lower-pressure pulmonary circulation rarely develops atherosclerotic plaques even in the face of elevated serum lipids (81). The chief manifestations of hypertensive injury in the heart include concentric left ventricular hypertrophy, congestive heart failure, atrial fibrillation, and coronary artery atherosclerosis, leading to increased risk of myocardial infarction (80–82). Additional cardiovascular risk is seen in hypertensive patients with the metabolic syndrome (83) defined as three or more of the following conditions: abdominal obesity, triglycerides greater than 150 mg/dL, high-density lipoprotein cholesterol less than 40 mg/dL in men or 50 mg/dL in women, blood pressure greater than 130/85 mm Hg or being treated for hypertension, and a fasting glucose greater than 110 mg/dL. Hypertension is associated with cerebral hemorrhage due to lesions in the penetrating vessels in the midbrain or due to rupture of berry aneurysms. Additional cerebrovascular complications of hypertension include stroke and vascular dementia (80). Aortic aneurysms as well as claudication are well-known peripheral artery manifestations of hypertension. Hypertension may cause retinal arteriolar thickening, hemorrhage, exudates, or papilledema (77). Hypertensive injury to the kidney is manifest chiefly by the appearance of microalbuminuria and/or decline in estimated glomerular filtration rate (eGFR) (84).

Accelerated Hypertension (Malignant Hypertension)

The current classification of hypertension no longer uses the term *malignant hypertension* (1). However, this term remains in the literature. In the past, malignant hypertension was defined as severe elevation of arterial pressure in combination with funduscopic changes including retinal hemorrhages and exudates with or without papilledema (85). It is often accompanied by target organ damage. It affects less than 1% of patients with essential hypertension, and its incidence has not declined since its description (86). The clinical symptoms may include visual disturbances, headache, headache with visual disturbance, heart failure, stroke or transient ischemic attack, or dyspnea (87). Hematuria is present in as many as 21% of patients, and some patients have gross hematuria. Significant proteinuria is present in 63.4% of patients and was associated with higher serum creatinine levels (87).

Malignant hypertension may occur without any history of hypertension, or it may be preceded by a period of essential hypertension. It may also complicate secondary forms of hypertension such as renal parenchymal disease, renal artery stenosis, various endocrinologic causes of hypertension, or cocaine abuse (86,88). Lane et al. (86) studied 446 patients (64.5% were men) with malignant hypertension who had been admitted to or were referred to City Hospital, Birmingham, England, between 1964 and 2006. Of these patients, 54.3%

had a history of essential hypertension. The remaining patients had secondary hypertension caused by renal artery stenosis, renal parenchymal diseases, or other such conditions. They found an incidence of 1 to 2 cases of malignant hypertension per 100,000 per year and found no apparent decline over the 40 years of the study. An excess of black and Asian patients has been noted relative to the number expected for the population studied in several cohorts (86,89). Survival has increased with the introduction of more effective antihypertensive drugs. In particular, patient survival has increased from 33% to 90% over the past 40 years (86) with 10-year renal survival of 84% (90). Factors involved in determining survival include creatinine at presentation and successful control of blood pressure and proteinuria during follow-up (86,90).

The cause of the switch from benign to malignant hypertension is not yet fully understood. Many authors have suggested that the renin-angiotensin axis may be paradoxically stimulated by an ischemic renovascular bed stemming from microvascular damage due to increased blood pressure (91,92). This theory has been supported by a transgenic model of malignant hypertension that has been developed by the insertion of the mouse *Ren-2* renin gene into Sprague-Dawley rats (93). The affected rats had increased blood pressure and typical renal changes of malignant hypertension and died at 50 to 90 days of age. Heterozygotes showed target organ damage in the heart and kidney but no other characteristics of malignant hypertension. This model has been further refined by inserting the *Ren-2* renin gene fused to the cytochrome P450 1a1 (Cyp1a1) promoter (94) that allows controlled inducible angiotensin II–dependent hypertension. Study of this model shows the expected pathologic findings of malignant hypertension and allows therapeutic manipulation. For example, administration of aliskiren, which directly inhibits renin, normalizes blood pressure in these rats (95). Patients with malignant hypertension have elevated plasma renin activity and aldosterone. These levels correlate to markers for intravascular hemolysis and renal function in patients with malignant hypertension (92).

Laboratory Findings Including Proteinuria

Hemolytic anemia is frequently seen in malignant hypertension, with schistocytes on the peripheral blood smear. Thrombocytopenia and negative results on Coombs test are also part of this hematologic picture, which is also known as microangiopathic hemolytic anemia or thrombotic microangiopathy (TMA). Other conditions associated with this condition include hemolytic-uremic syndrome, thrombotic thrombocytopenic purpura, scleroderma renal crisis, and various drug-related injuries. These conditions are described in Chapters 18 and 25. Hyperuricemia has been associated with an increased risk in the future for hypertension particularly in the young and in women independent of other risk factors (96).

Most of the abnormal laboratory values in patients with hypertension reflect end-organ damage. When microalbuminuria is defined as 20 to 200 μg/min (30 to 300 mg/24 hours), the prevalence of microalbuminuria ranges between 5% and 60% in hypertensive patients (97,98). The microalbuminuria correlates to the level of arterial pressure. Furthermore, microalbuminuria is predictive of renal damage, cardiovascular disease, and death in hypertensive patients (80,97,99,100). Wang et al. (101) examined the urinary albumin-to-creatinine ratio in 1499 nonhypertensive individuals. During a mean follow-up period of 2.9 years, 15% of these persons developed hypertension and another 33% progressed to a higher level of hypertension. The authors found that urinary albumin excretion (UAE) predicted these changes in blood pressure even at levels lower than the conventional threshold for microalbuminuria. Microalbuminuria arises in part due to endothelial dysfunction as is discussed below. Microalbuminuria is also secondary to changes in glomerular hemodynamics due to loss of autoregulation. Ten percent of patients at the onset of their hypertension have hyperfiltration (99).

Biomarkers other than microalbuminuria have also been studied in hypertension. The biomarkers studied fall into the categories of markers of endothelial dysfunction, oxidative stress, and inflammation. Endothelial damage is known to occur and may precede the elevated UAE (97). Microalbuminuric essential hypertensive patients had higher serum levels of circulating endothelin-1 (ET-1) and basic FGF (102). Furthermore, there was a correlation between these factors and the level of microalbuminuria. Pedrinelli et al. (103) examined levels of von Willebrand factor (vWF) in 10 hypertensive patients with microalbuminuria and compared them with 10 hypertensive patients without microalbuminuria and 10 controls without microalbuminuria. These investigators found that vWF was increased in the serum of patients with microalbuminuria. Furthermore, serum levels of vWF correlated with UAE, mean blood pressure, and age. Mean blood pressure and vWF both contributed to the rise in UAE when multiple regression analysis with UAE as the dependent variable was performed. Patients with microalbuminuria had higher serum creatinine levels and lower creatinine clearance than did hypertensive patients without microalbuminuria. An inverse correlation was found between vWF and creatinine clearance. The authors concluded that blood pressure may contribute to endothelial damage and thereby may promote microalbuminuria. Other investigators found that serum levels of both intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 were increased in patients with essential hypertension as compared to controls (104). Furthermore, the levels were highest in such patients with microalbuminuria. Additional markers of endothelial dysfunction include inhibitors of nitric oxide (NO) such as plasma asymmetric dimethylarginine and homocysteine. Markers of inflammation such as C-reactive protein (CRP) and plasminogen activator inhibitor-1 may also be elevated. Any of these indicators may be found in patients with hypertension and even in some patients with white coat hypertension (105). Of further interest is that the markers of inflammation including CRP and tumor necrosis factor–α may be present in patients with prehypertension (106,107).

Biomarkers can also be used for risk stratification for end-organ damage, especially the heart, due to hypertension. A recent systematic review and meta-analysis showed that elevated levels of matrix metalloproteinases (MMP)-2 and 9 as well as tissue inhibitor of MMP (TIMP) are present in hypertensive patients (108). MMP-9 and TIMP are elevated in hypertensive patients prior to the onset of heart failure and may be good markers of potential risk for end-organ damage. TIMP has higher levels in patients with left ventricular hypertrophy, indicating the presence of myocardial remodeling. Serum MMP-2 is elevated to a greater degree in hypertensive patients with diastolic heart failure (108). Other useful biomarkers of ventricular remodeling include atrial natriuretic peptide and brain natriuretic peptide (109).

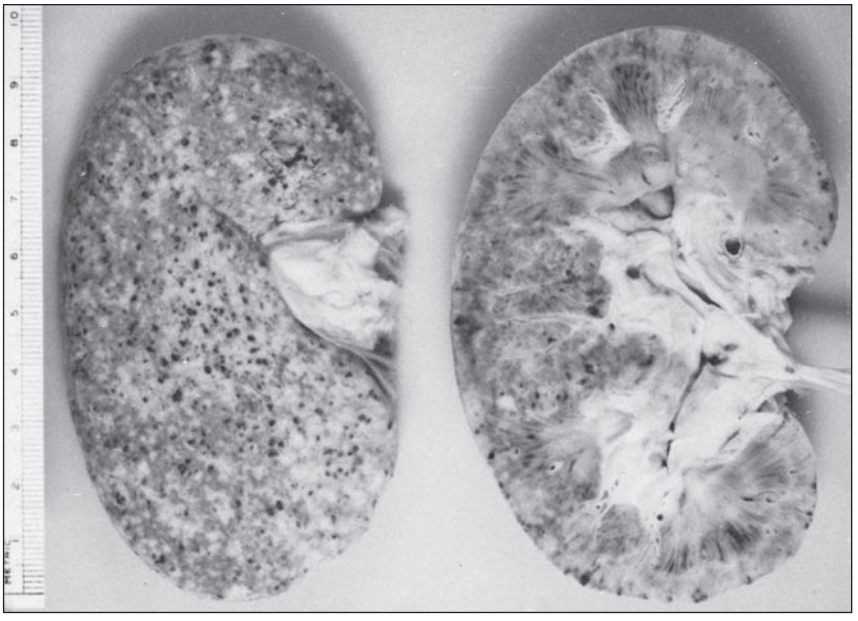

FIGURE 20.1 Kidney from a patient who had the malignant phase of essential hypertension. Kidney weights: left, 150 g; right 130 g. Note numerous petechial hemorrhages.

Pathologic Findings

Gross Pathology

The kidneys in benign nephrosclerosis are typically reduced in size, with a total weight ranging between 120 and 250 g (normal = 300 g total weight). The two kidneys are usually affected equally. The capsular surface is most commonly finely granular, reflecting disease in small arteries and arterioles. The granules are formed by zones of relatively preserved or even hypertrophied renal parenchyma alternating with neighboring nephrons with diseased arterioles, which are scarred and depressed. When larger renal arteries show intimal thickening and damage larger clusters of nephrons, however, coarser granularity may be superimposed against a background of fine granularity with scattered V-shaped pits. Such larger scars will take the form of pits, which, on cut surface, extend only through the cortex and not the medulla, indicating their relationship to the interlobular or arcuate vessels. These features differentiate them from pyelonephritic scars, which are larger and U shaped, and extend through a fibrotic medulla to end in a dilated, distorted, and inflamed calyx. The capsule may strip with difficulty over such pitted scars. The kidney may be difficult to cut because of the interstitial fibrosis. The cut surface also reveals cortical thinning. Glomeruli may be difficult to identify. Atherosclerosis may be evident in the main renal artery or in any of its branches. Cortical cysts or small adenomas may also be present. If the patient has a short history of hypertension or only mild elevations, there may not be any gross alterations.

The gross appearance of the kidney from an untreated patient with accelerated hypertension differs from that of a patient who has been treated or who has had a period of less severe hypertension preceding the onset of malignant hypertension. In the untreated patient, the combined kidney weights can range from 130 to 410 g (91). The capsular surface may be smooth in those patients without preceding hypertension but may show a granular surface for those with long-standing hypertension. Petechial hemorrhages, frequently representing congested glomeruli sometimes with hemorrhage into local tissue, are prominent and may also be seen on the cut surface. Small infarcts may also be noted (Fig. 20.1). In patients who have been treated, the petechial hemorrhages and infarcts are less prominent or not visible at all.

Light Microscopy

Glomeruli

The glomeruli may be normal or may simply show age-related changes (110). A few globally sclerosed glomeruli are seen in all adults despite the absence of increased blood pressure. Sclerosed glomeruli may disappear and sometimes one may find such "disappearing glomeruli" that appear to merge into the surrounding renal parenchyma (Fig. 20.2). In hypertensive nephropathy two additional types of glomerular changes are seen: ischemic glomeruli and solidified ones (111). The earliest change is that of collapse of the capillary loops with apparent thickening of their walls on hematoxylin and eosin

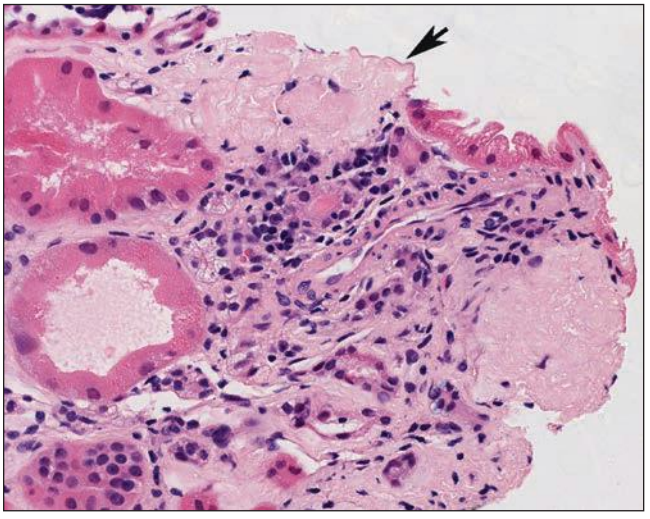

FIGURE 20.2 Disappearing glomerulus (*arrow*) merging into surrounding tissue. (H&E, ×260.)

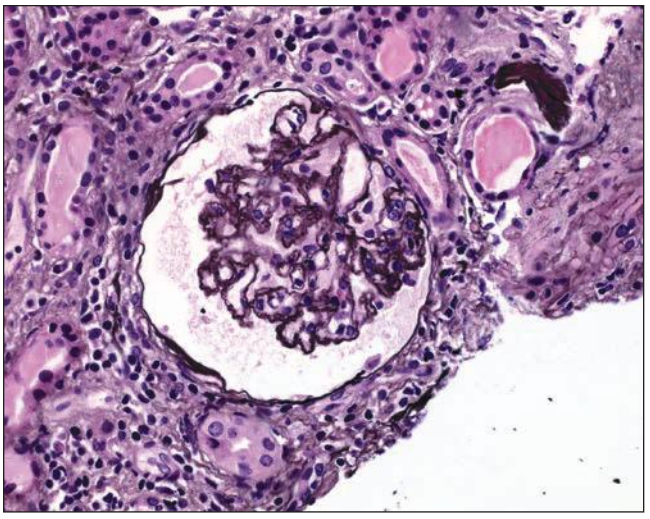

FIGURE 20.3 Glomerulus with wrinkling of the GBM accompanied by reduction of capillary lumen diameter. (PAS-Jones, ×390.)

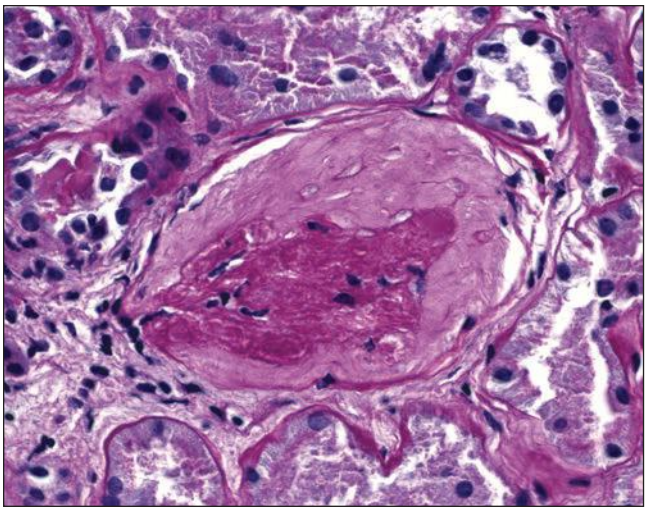

FIGURE 20.5 The glomerular tuft is shrunken with wrinkling of the capillary walls. The capsular space contains pale-staining material. The PAS technique clearly distinguishes between the collapsed tuft and the collagenous material occupying the capsular space. (PAS, ×340.)

(H&E)-stained sections. Periodic acid-Schiff (PAS) reaction or silver stain, however, shows that the apparent thickening is chiefly due to wrinkling of the capillary basement membrane (Fig. 20.3). With time, the entire glomerular tuft shrinks and retracts to the vascular pole. This is accompanied by the filling in of the Bowman space by a faintly eosinophilic material. H&E-stained sections of such glomeruli reveal only pink, nondescript balls (Fig. 20.4). However, PAS staining demonstrates the shrunken tuft as well as mild thickening of the Bowman capsule (Fig. 20.5). Furthermore, the material filling the Bowman space is PAS negative, a characteristic staining pattern for collagen. This change may begin in the hilar portion of the tuft, but it soon fills the entire space emptied by the shrinking glomerulus (112) until it becomes obsolescent.

The solidified glomerulus is characterized by an increase in the mesangial matrix, which results in either segmental or global solidification (sclerosis) of the glomerular tuft (Fig. 20.6), extending to the Bowman capsule without collagenization of the Bowman space. Such glomeruli frequently contain hyalinosis lesions and represent "decompensated benign nephrosclerosis" (DBN) (113,114). This term is used by Bohle et al. to describe those cases of benign nephrosclerosis in which many glomeruli show prominent hyalinosis accompanied by mesangial widening. In a study of hypertensive patients, these authors found that 775 kidneys showed the obsolescent glomeruli described above accompanied by hyaline arteriolosclerosis. An additional 251 patients had the solidified glomeruli of DBN (114). These latter patients developed chronic renal failure within a few years and had higher levels of blood pressure, proteinuria, and higher

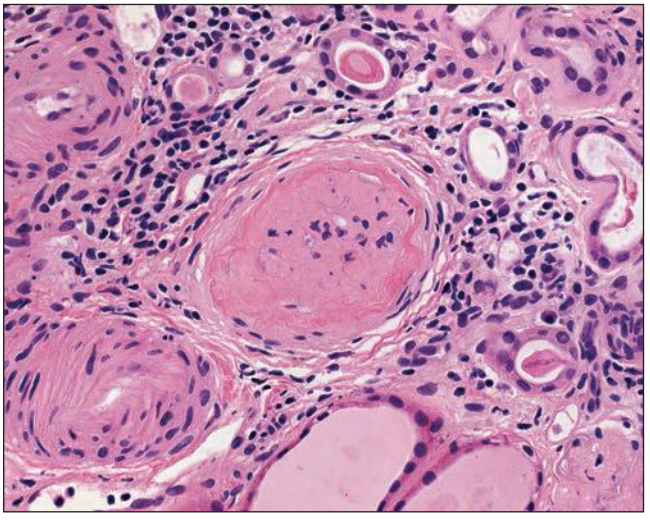

FIGURE 20.4 Sclerotic glomerulus with obliterated capillary loops. (H&E, ×260.)

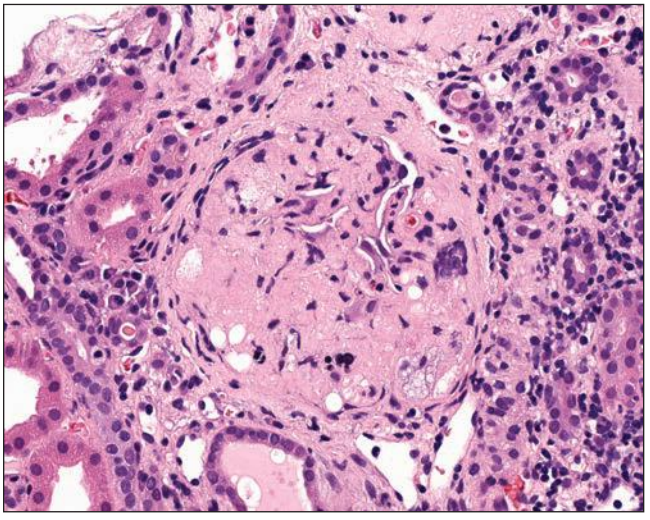

FIGURE 20.6 Solidified glomerulus extending to the Bowman capsule without collagenization of the Bowman space. Foam cells may be seen within the glomerulus. (H&E, ×260.)

serum creatinine than patients with solely ischemic lesions. Solidified glomeruli are readily distinguished from obsolescent glomeruli by use of the PAS staining technique. Marcantoni et al. (74) recognized these solidified glomeruli of DBN in a higher proportion of African American hypertensive patients than in Caucasians. They also reported a higher frequency of segmental sclerosis in the patients with DBN. Thus, this alteration may represent a secondary form of focal segmental glomerulosclerosis (FSGS). Kincaid-Smith (115) has suggested that the increase in segmental glomerular lesions may be related to the increase in the association between the metabolic syndrome (chiefly obesity and insulin resistance) and hypertension. She posits that these factors are producing the segmental glomerular sclerosis rather than the hypertension. Kidneys with hypertensive nephrosclerosis also may contain a population of hypertrophied glomeruli (116). Hill et al. (116,117) have described pathologic differences in the afferent arterioles supplying these different types of glomeruli. This is addressed below in the discussion of the pathogenesis of these changes.

Several other glomerular alterations have been noted in the kidneys from hypertensive patients. First, the podocyte number is decreased in hypertensive nephrosclerosis accompanied by increased urinary podocin, nephrin, and synaptopodin as compared to healthy controls (118). Reduced podocyte numbers have been described in a number of renal diseases with glomerulosclerosis. Hoy et al. (119) have demonstrated lower glomerular number in hypertensive American whites and Australian Aborigines. However, there was no difference in nephron number in hypertensive blacks from the United States as compared to nonhypertensive blacks. However, the mean glomerular volume was greater in hypertensive blacks as compared to nonhypertensive controls (119). Finally, the juxtaglomerular apparatus (JGA) is slightly enlarged with respect to numbers of cells in patients with benign hypertension compared with normotensive controls (120).

The glomerular changes in accelerated (malignant) hypertension may be acute or chronic (121–123). They are identical to those seen in secondary forms of malignant hypertension in association with a preexisting renal parenchymal disease; the alterations described here are superimposed on the changes of the respective condition. The acute changes are focal; most glomeruli appear unchanged. The most obvious change is that of fibrinoid necrosis, which is usually segmental (Fig. 20.7). This lesion is eosinophilic, but it is most easily seen with a silver stain, which demonstrates interruption of the glomerular basement membrane (GBM). Furthermore, the intense eosinophilia of the area of necrosis stands in sharp contrast to the black staining of the GBM and mesangial matrix (Fig. 20.8). The mesangial matrix may also show loosening, so-called mesangiolysis. The fibrinoid necrosis may extend from the afferent arteriole, or it may be peripheral and associated with a crescent. Fibrin, which accounts for the eosinophilia of the lesion, may also be seen in other capillary lumina within the glomerular tuft or may accumulate under endothelial cells. Endothelial cells of arterioles and capillaries may become swollen and obscure the lumen. Fibrin platelet thrombi with red blood cell fragments may also be seen (Fig. 20.9). In other glomeruli, the predominant alteration is one of intense congestion with dilation of the capillary lumina (Fig. 20.10). The final acute alteration is that of thickening of capillary walls in open capillary lumina. Silver stain demonstrates double

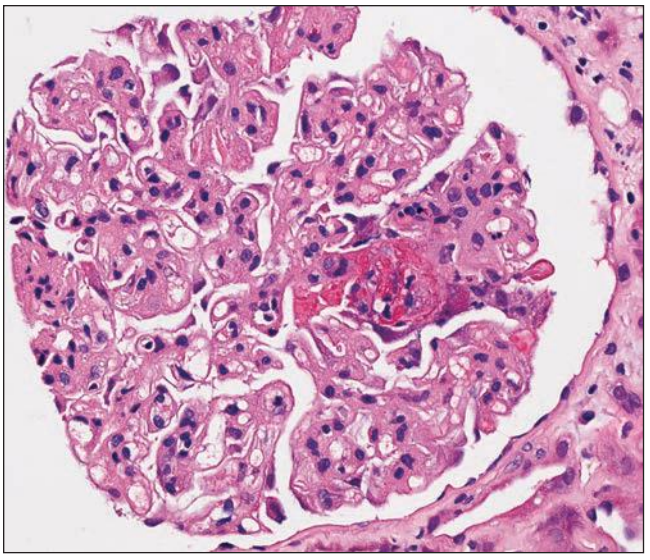

FIGURE 20.7 Glomerulus with segmental necrotizing lesion (fibrinoid necrosis) characterized by eosinophilia, karyorrhexis, and apparent disruption of glomerular architecture. (H&E, ×260.)

contours indicative of subendothelial widening and new basement membrane formation (Fig. 20.11) (see also Chapter 18.)

The chronic changes are of two different types. The first is similar to that seen in benign hypertension, in that there is collapse of the tuft with wrinkling of the GBM accompanied by collagenization of the Bowman space. This change may occur with or without a preceding period of benign hypertension, although it is much more prominent in patients with a history of hypertension. In the second type, glomeruli are also collapsed, but seem almost acellular and, on silver stain, show the subendothelial widening described earlier in the preceding paragraph. Thus, the collapsed glomeruli have evolved from those that showed subendothelial widening.

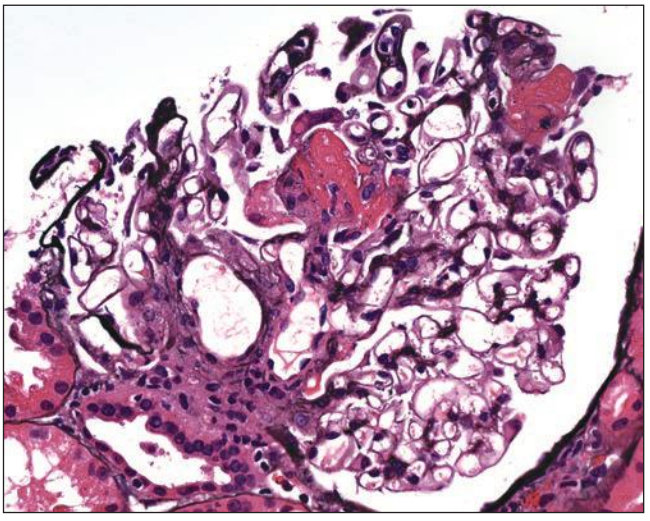

FIGURE 20.8 Fibrinoid necrosis involving several lobules in the center and upper right of the tuft, with segmental disruption of the basement membrane at the central portion. (PAS-Jones, ×260.)

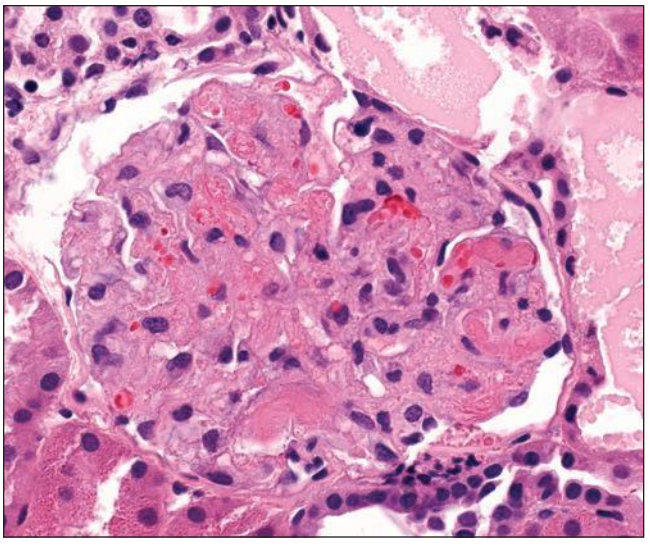

FIGURE 20.9 Glomerulus with dilated capillaries filled with red cells, red cell fragments, and amorphous material probably representing platelets and fibrin. An identical picture is seen in other conditions with TMA such as hemolytic-uremic syndrome and scleroderma. (H&E, ×520.)

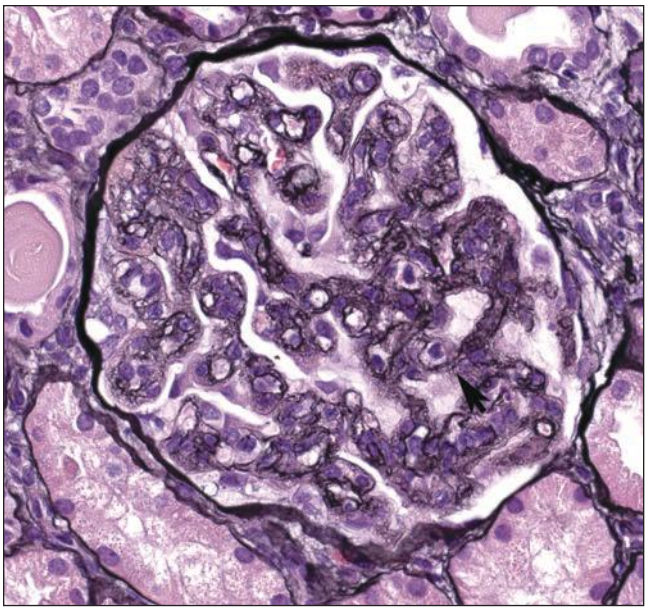

FIGURE 20.11 Glomerulus with double contours (*arrow*) representing duplication of the GBM and subendothelial widening. (PAS-Jones, ×370.)

Tubules

The tubules in either benign or malignant hypertension may be atrophic and sometimes contain hyaline casts. The epithelial cells are flattened and surrounded by a thickened tubular basement membrane, which may be wrinkled. Such atrophic areas often alternate with zones of tubules with dilated lumina and tall hypertrophied epithelial cells (Fig. 20.12). Such zones form the granularity seen on the capsular surface of gross specimens. The size of the scars, or fields of atrophic tubules, depends on the diameter of the narrowed vessel supplying the area, thus determining the number of nephrons affected. Although such atrophic tubules are frequently near an obsolescent glomerulus, this is not always the case, a finding that may reflect the greater susceptibility of tubules to ischemia. Actual loss of tubules may also occur suggested by glomerular crowding. If the patient has proteinuria, protein reabsorption droplets may be present in the tubular epithelial cells. In patients with sudden onset of malignant hypertension, patchy necrosis of tubules may be evident, and there may be cortical infarcts. Such areas are more common in scleroderma-associated TMA than in that associated with malignant hypertension (see also Chapter 18.)

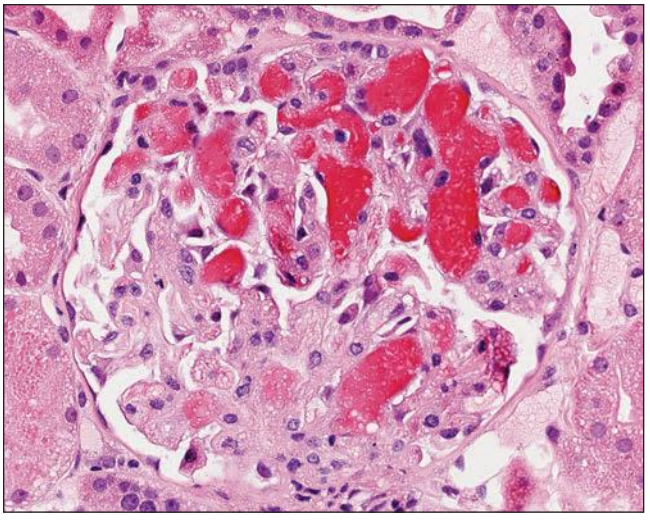

FIGURE 20.10 Glomerulus with congested capillary loops. (H&E, ×260.)

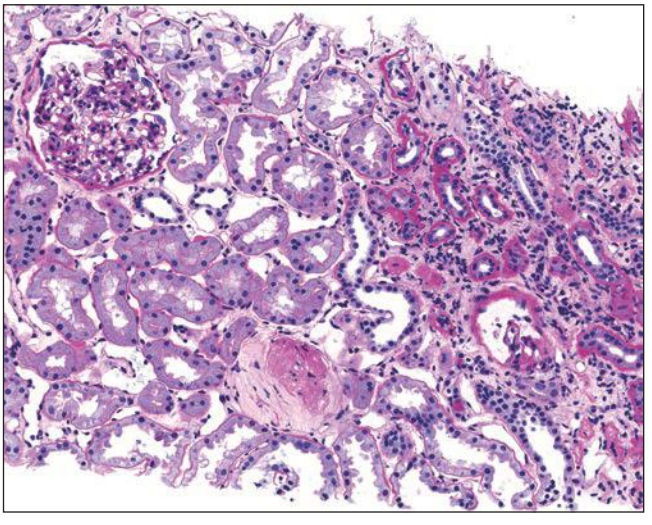

FIGURE 20.12 Renal biopsy showing the zone of atrophic tubules and interstitial fibrosis to right with normal tubules and the interstitium to left. Some of the normal tubules show mild dilation. (PAS, ×125.)

Interstitium

The interstitium is widened in areas with atrophic tubules. Increased collagen is noted. Chronic inflammatory cells, usually small lymphocytes, may be widely dispersed in the areas of scarring; however, larger aggregates of lymphocytes may be present in the scars in subcapsular zones. Mast cells are increased in the interstitium early in hypertensive nephropathy (124). Morphometric studies by Grund et al. (125) have shown a correlation between the amount of interstitium and the serum creatinine level. These authors hypothesized that the renal intertubular capillaries had diminished cross-sectional area resulting from interstitial fibrosis and that this change may have contributed to the decrease in the GFR. They did not determine whether the fibrosis preceded the loss of capillaries or vice versa. The chronic hypoxia hypothesis as a model for the progression of renal disease was first suggested by Fine et al. (126). One of the mechanisms for this is damage to the tubules due to reduced blood flow through the peritubular capillaries (111). The mechanisms are further discussed below. The changes in accelerated hypertension are similar to those seen in benign nephrosclerosis.

Blood Vessels

The changes vary with the size of the vessel involved and also differ among individual patients. Arcuate and larger arteries show the alterations typical of atherosclerosis, as manifest by fibrous intimal thickening that results in reduction of the vessel lumen. The internal elastic lamina may show splitting, which is best seen with either a silver or elastic stain. This change is different from the abrupt breaks seen in vasculitis. Lipid-containing macrophages are not usually evident except in the intima of the main renal artery or its branches. In cases in which malignant hypertension is primary, and not secondary to a period of lower levels of hypertension, the larger arteries may not show any alteration. Otherwise, the arteries are similar in appearance to those seen with any level of hypertension.

Interlobular arteries show fibroelastic intimal thickening with reduplication of the internal elastic lamina (Fig. 20.13). The degree of intimal thickening in arteries is closely linked to this change in arterioles (127). The media may be thickened in small arteries and/or arterioles (Fig. 20.14), reflecting hypertrophy, hyperplasia, or remodeling. In any case, lumen size is decreased, and this decrease may be the result of remodeling, alterations in stiffness or compliance, and functional abnormalities. Remodeling and functional changes are discussed below. Stiffness may be dependent on alterations in the amount and type of collagen, the collagen-elastin ratio, and the presence of proteoglycans (128). The latter has been described in humans. An abnormal interaction among these elements may also lead to increased stiffness (128). In patients with accelerated hypertension, the smaller of the interlobular arteries may also show fibrinoid necrosis, which is a segmental change causing localized destruction of a vessel (Fig. 20.15). However, thickening of the intima with mucoid matrix and widely spaced, concentrically arranged cells are more typical changes (Figs. 20.16). The matrix has sparse collagen in a basophilic edematous-appearing

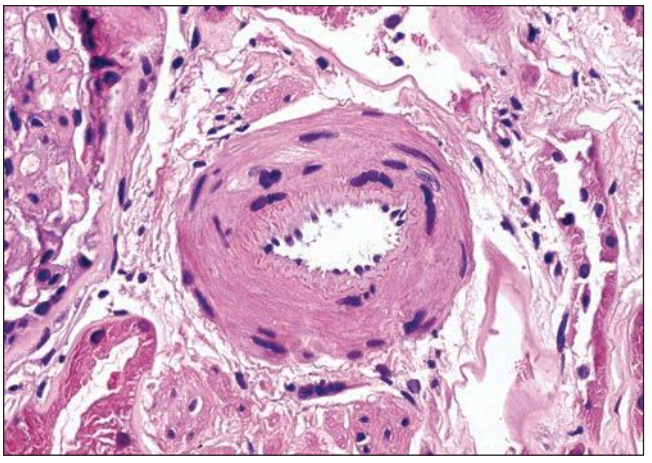

FIGURE 20.14 Small artery showing medial thickening with multiple layers of smooth muscle cells and wrinkling of the internal elastic lamina. (H&E, ×325.)

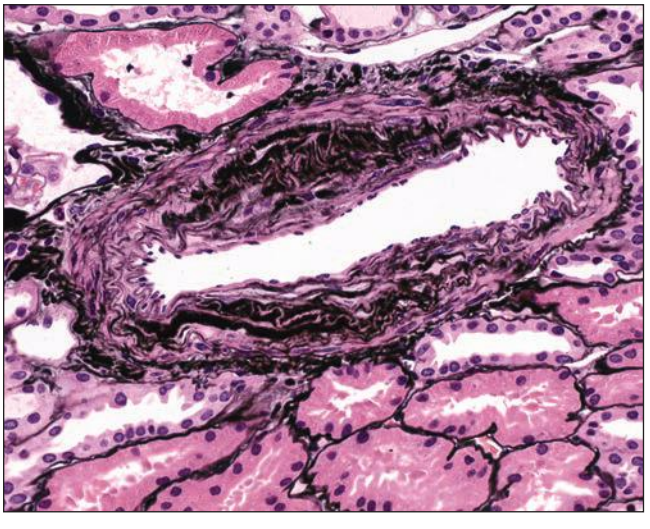

FIGURE 20.13 Interlobular artery with reduplication of the internal elastic lamina. (PAS-Jones, ×260.)

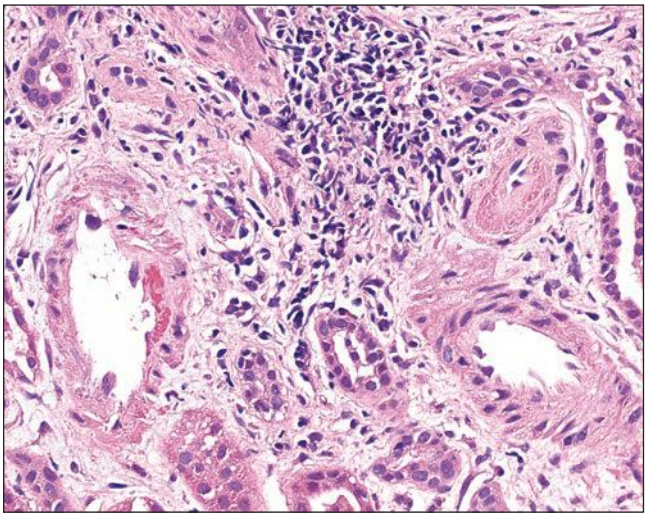

FIGURE 20.15 Small artery to left showing segmental fibrinoid necrosis characterized by intense eosinophilia and granular texture. Arteriole at upper right shows fibrinoid necrosis with near occlusion of the lumen. (H&E, ×260.)

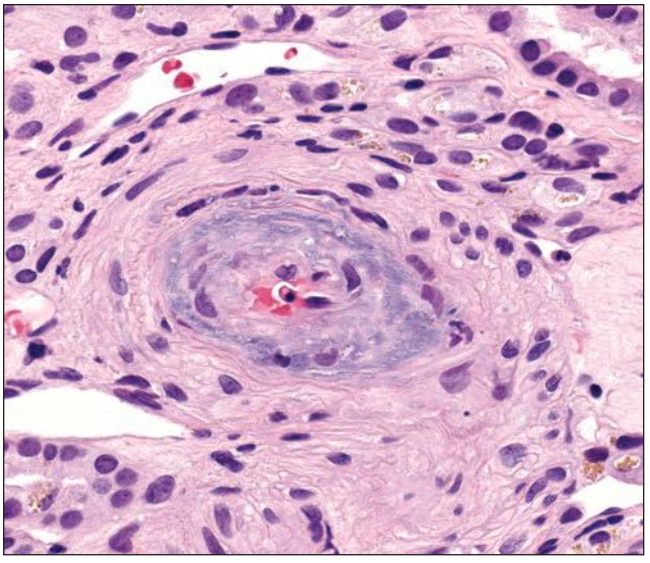

FIGURE 20.16 Small artery with mucinous basophilic intimal thickening. (H&E, ×470.)

matrix. This thickened intima may also show lipid-containing macrophages as well as reduplication of the elastic lamina (Fig. 20.17). Elastic reduplication is particularly prominent in those patients who had a prolonged period of benign hypertension before the onset of malignant hypertension. In these cases, the intimal thickening is luminal to the elastic reduplication. The intimal thickening extends along most of the length of the vessels and causes marked narrowing of the lumen that results in the devastating ischemic alterations that can cause renal failure. The most common terms used to describe this lesion are *onionskin thickening, endarteritis fibrosa*, and *musculomucoid intimal thickening*. These alterations in the kidney in malignant hypertension, including the findings of fibrinoid necrosis of

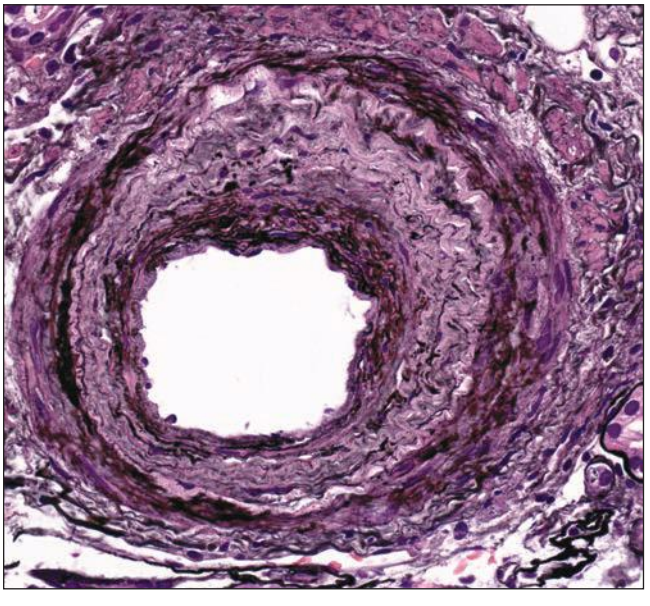

FIGURE 20.17 Interlobular artery with marked reduplication of the internal elastic lamina. (Periodic acid-methenamine silver, ×170.)

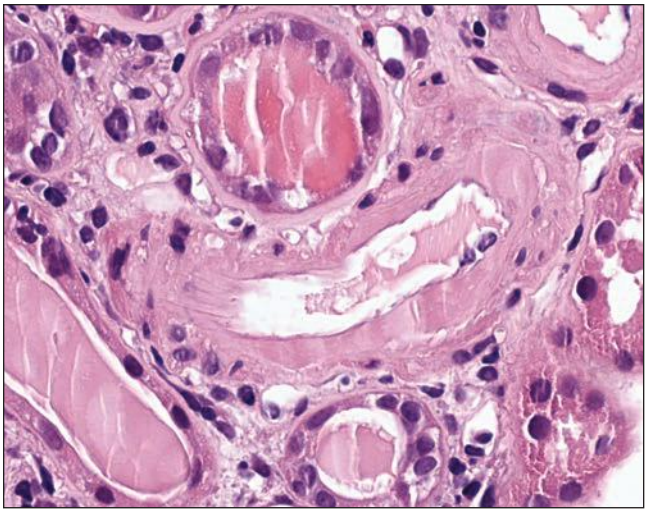

FIGURE 20.18 Arteriole with circumferential intimal hyalin deposition. Note glassy appearance. (H&E, ×520.)

the afferent arteriole, glomerulitis, and onionskin thickening of small arteries, were first described by Volhard and Fahr (129).

Arterioles are commonly affected by hyaline arteriolosclerosis, a change that is also present in the aging kidney. Hill (111) prefers the term arteriolar hyalinosis as the hyaline deposits occur in areas of loss of smooth muscle or arteriomalacia. Burchfiel et al. (130) found that the risk of arteriolar hyalinization increased with increases in diastolic blood pressure. The severity of arteriolar hyalinization also correlates with the degree of larger artery intimal thickening (131). This lesion is characterized by the presence of homogeneous eosinophilic material, sometimes with lipid in the subendothelium of the arterioles (Fig. 20.18). It may extend into the media and is more easily seen using the PAS stain (Fig. 20.19). Use of the

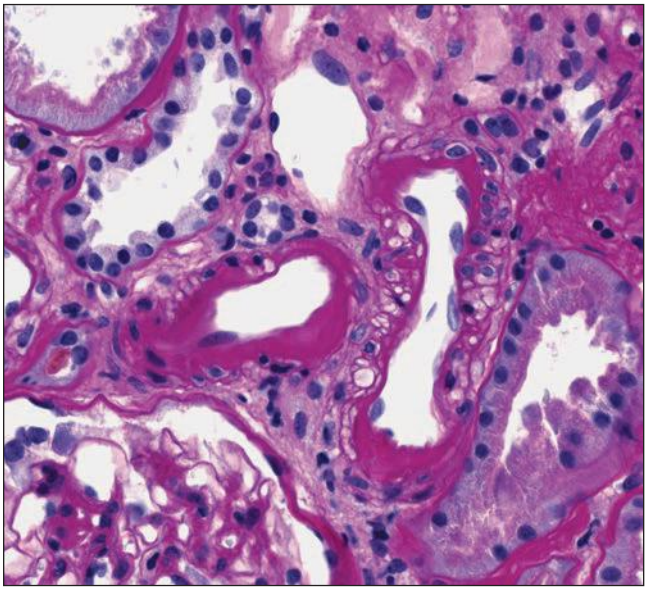

FIGURE 20.19 Small artery with intimal hyalin and arteriole to left with intimal deposition of hyalin extending toward the media and compressing smooth muscle cells. (PAS, ×400.)

term *hyalin* suggests that it appears glassy, to distinguish it from the more granular texture of fibrinoid necrosis, a lesion typical of the malignant phase of hypertension discussed below. In a morphometric study, Hill et al. (116) noted that arteriolar hyalinosis increased in frequency in hypertensive kidneys as compared to age-matched controls without hypertension. Furthermore, these vessels had larger luminal diameters and an increase in vascular smooth muscle and the extracellular matrix, resulting in a larger outer circumference. The afferent arterioles with hyalinosis were associated with either hypertrophied glomeruli or glomeruli with FSGS. These changes reflect the loss of autoregulation that may occur with hypertension resulting in transmission of elevated pressure through the dilated vessels and on to the glomerulus.

The characteristic lesion of severe (malignant) hypertension is fibrinoid necrosis. Eosinophilic material with a granular texture supplants the medial smooth muscle cells with loss of cell nuclei (see Fig. 20.15). This granular material must be differentiated from the glassy appearance of arteriolar hyalinosis, the more typical lesion of benign hypertension (see Fig. 20.18). Of course, arteriolar hyalinosis may also be present in patients with malignant hypertension, but the identification of true fibrinoid necrosis suggests the diagnosis of the accelerated phase. Fibrinoid necrosis also impinges on the vascular lumen. Areas of fibrinoid necrosis can be distinguished easily with the use of the PAS/Jones (silver) and the recognition of the granular texture rather than the glassy appearance of hyalin. The elastic lamina of the vessel wall as well as the interstitium between smooth muscle cells stain black from the silver. This finding is in sharp contrast to the bright pink staining of the fibrinoid material. Masson trichrome stain also delineates fibrinoid necrosis well as the fibrin stains bright red (Fig. 20.20). Thrombus formation is often superimposed on the area of necrosis, resulting in complete occlusion of the vessel. Fragmented red blood cells are frequently seen within either the thickened intima or the vessel wall, evidence of TMA

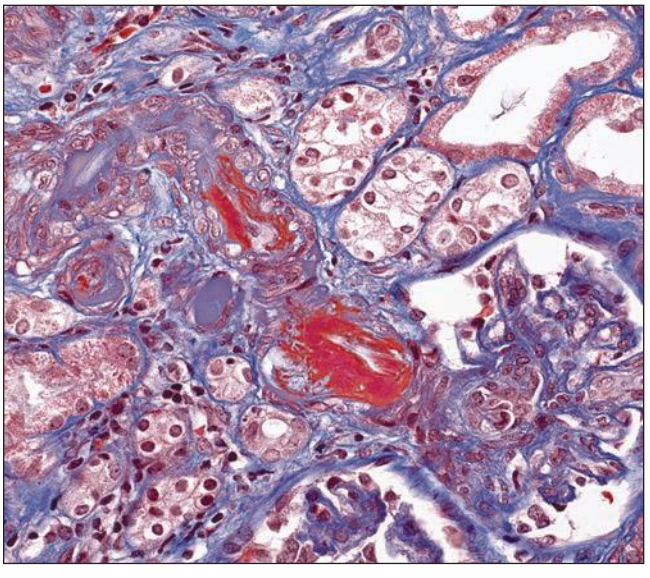

FIGURE 20.20 Afferent arteriole with fibrinoid necrosis staining *bright red*. The lumen is nearly totally occluded, resulting in collapse of the glomerulus being fed by the vessel. (Masson trichrome, ×310.)

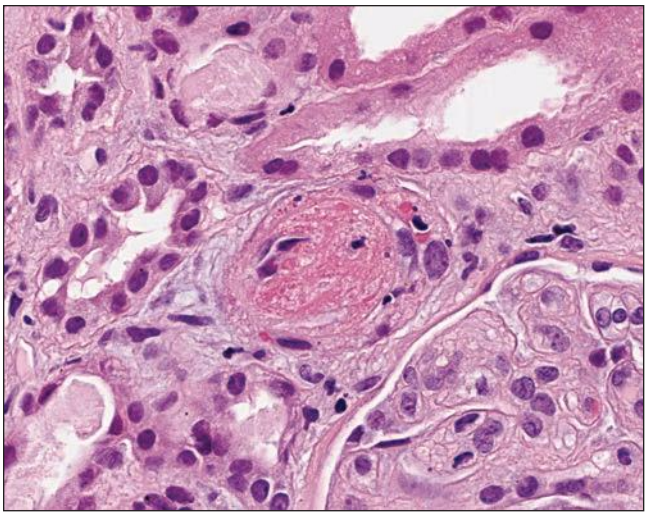

FIGURE 20.21 Arteriole showing TMA with platelet-fibrin thrombus occluding the lumen. Also note fragmented red blood cells within the vessel wall. (H&E, ×520.)

(Fig. 20.21). Mixed inflammatory cells are occasionally present, but their presence is not necessary to make the diagnosis.

Hughson et al. (132) in a study of patients with malignant nephrosclerosis identified nodules in small arteries and arterioles. These nodules were composed of spindled cells with a vascular network similar to the plexiform lesion seen in pulmonary hypertension. He hypothesized that the pathogenesis was due either to arterial necrosis or organization of thrombi or to a combination of the two, with the necrosis occurring first followed by the thrombus. Hughson also suggested that the intimal hyperplasia was due to turbulent blood flow. These changes were seen most commonly in the kidneys, followed by the periadrenal fat, pancreas, intestines, gallbladder, and heart.

Thrombotic Microangiopathy and Malignant Nephrosclerosis

The term *malignant nephrosclerosis* was first used by Fahr (133) in a description of the renal pathologic characteristics of a patient who had an accelerated or malignant form of hypertension. Bohle et al. (134) examined the issue of malignant nephrosclerosis in great detail. They divided their patients into two groups: one with the changes of fibrinoid necrosis in glomeruli and arterioles and the other with onionskin alterations in the intima of arteries. They designated the first group with either normal blood pressure or recent onset of hypertension as primary malignant hypertension. Bohle et al. believed that in these cases, the renal vascular morphologic changes preceded the hypertension. The other group had long-standing accelerated hypertension and was considered secondary malignant hypertension. The authors believed that the vascular changes in these cases were caused by the hypertension. Patients in the primary malignant hypertension group were younger than those in the secondary group. Furthermore, women outnumbered men, and the primary group had more severe hemolytic anemia and often presented in acute renal failure. The changes are those of TMA (discussed in Chapter 18) and are the same as seen in hemolytic-uremic syndrome,

thrombotic thrombocytopenic purpura, renal involvement in systemic sclerosis (scleroderma), toxicity of certain drugs such as cyclosporine and mitomycin C, and complications of radiation and bone marrow transplantation. It is characterized by fibrinoid changes in both glomeruli and renal arterioles accompanied by fragmented erythrocytes (see Fig. 20.20). The glomeruli may also show collapse and wrinkling of the tuft. The renal arterioles and small arteries show intimal thickening, often with a mucoid appearance resulting in marked narrowing of their lumina. As suggested in the name, thrombotic occlusion of the lumen is often superimposed on this narrowing.

The question of the primacy of malignant levels of hypertension in the pathogenesis of these microangiopathic lesions is still controversial. Most of the cases I see now that have the changes I consider representative of malignant nephrosclerosis are superimposed on the kidneys with primary glomerular disease. In these patients, severe renal parenchymal disease is present, and the history is consistent with preexistence of the glomerular disease before the onset of the malignant levels of hypertension. Thus, they can be considered as cases of secondary malignant hypertension. However, I have also seen a patient with IgA nephropathy and the changes of malignant nephrosclerosis in whom glomerular disease was not severe and the tubules and interstitium showed only focal fibrosis and atrophy. Nevertheless, the arteries showed changes consistent with the malignant hypertension, which was present clinically. In that case, I believe that the malignant hypertension was separable from the renal parenchymal disease and was thus primary or essential malignant hypertension. In most cases with microangiopathic changes, malignant hypertension is prominent clinically. However, I have seen the alterations of TMA without documentation of malignant levels of hypertension. In one particularly memorable case of scleroderma renal crisis, the patient never had a recorded blood pressure exceeding 120/80 mm Hg yet had spectacular fibrinoid necrosis of both glomeruli and arterioles.

Immunofluorescence Findings

Arterioles with hyaline accumulation have been shown to contain IgM and C3 most commonly, but IgG, IgA, IgE, and fibrinogen have also been recorded (135) (Fig. 20.22). C3 may also be observed without accompanying immunoglobulins. The most common reactant seen in immunofluorescence studies of malignant hypertension is fibrinogen (134). It may be noted in areas of fibrinoid necrosis, as would be expected, but it is also seen in glomerular capillary loops and in larger vessels without obvious fibrinoid necrosis. These changes are more prominent in the more active cases. On occasion, other immunoglobulins can be identified, presumably accumulating nonspecifically in areas of injury.

Electron Microscopy

Ultrastructural studies (122,136,137) of glomeruli from patients with hypertension demonstrated wrinkling and thickening of the GBM, which also becomes denser (Fig. 20.23). Electron microscopy confirms the presence of collagen fibrils with scattered cells within the capsular space and reduplication of the basement membrane material of the Bowman capsule (Fig. 20.24). The presumption has always been that the shrinkage of the tuft results from ischemia (136,137), but no good explanation has been advanced for the accumulation of

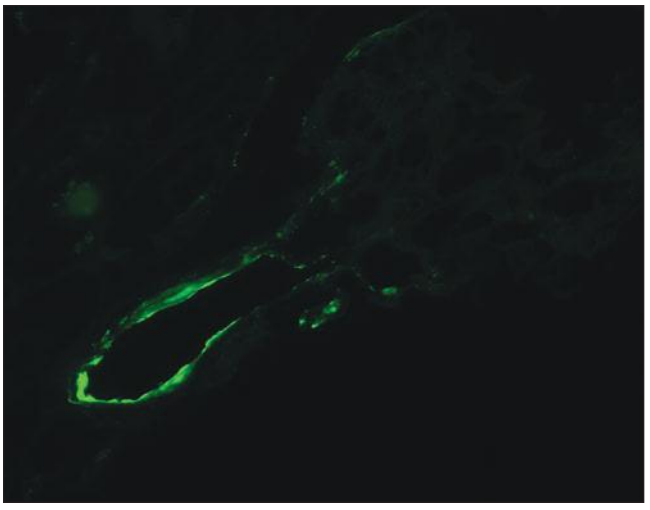

FIGURE 20.22 Immunofluorescence micrograph of afferent arteriole staining with C3 antiserum. (C3 antiserum, ×260.)

collagenous material in the Bowman space. In open glomeruli, one may see mild but widespread subendothelial widening sometimes with new basement membrane formation (Fig. 20.25).

Ultrastructural studies of intimal thickening of larger arteries in hypertension show that the intimal cells are myofibroblasts as characterized by both abundant rough endoplasmic reticulum and myofibrils distributed along the periphery of the cell. Immunohistochemistry with anti-smooth muscle–specific actin confirms the smooth muscle differentiation of these cells as well. Electron microscopy also demonstrates the presence of extracellular proteoglycans (small granules), which are responsible for imparting the mucoid appearance to the thickening (122,138).

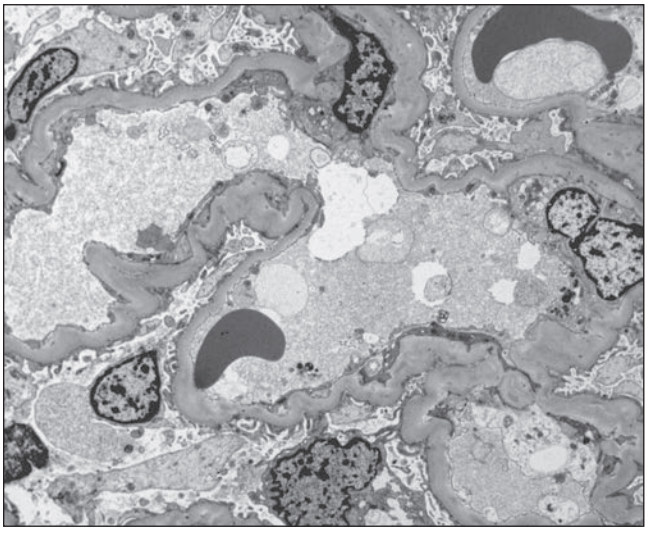

FIGURE 20.23 Electron micrograph showing wrinkling of the GBM with variability in its thickness. Foot processes are coarsened, but frank effacement is not seen. (Uranyl acetate and lead citrate, ×3600.)

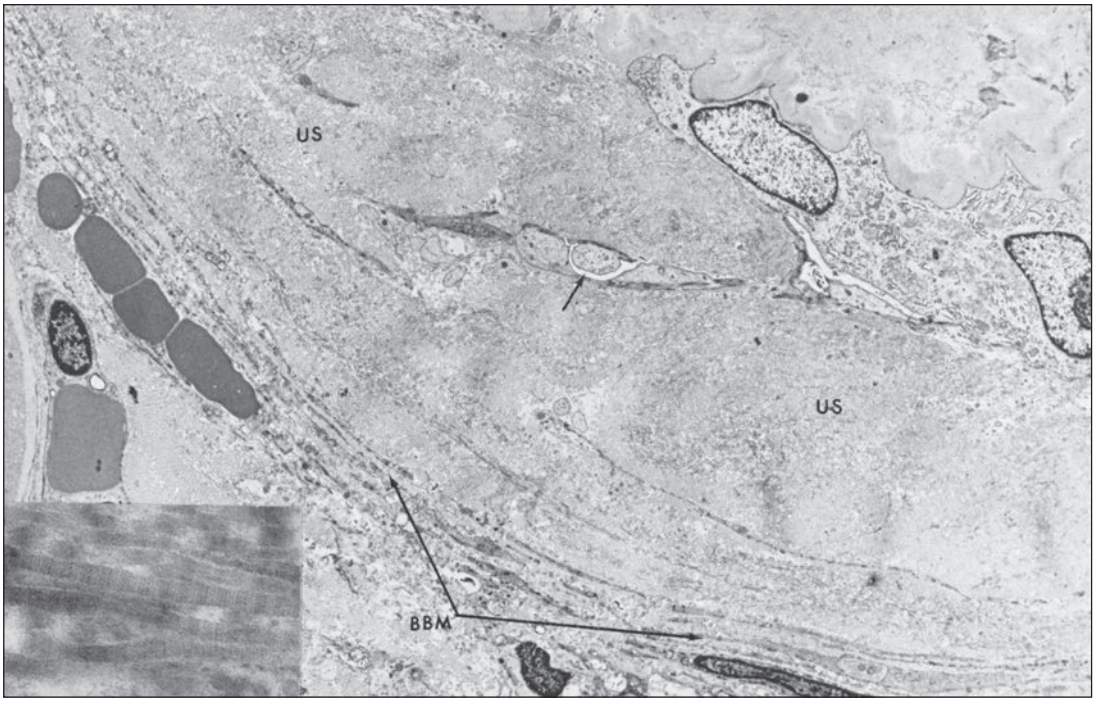

FIGURE 20.24 **Electron micrograph from an obsolescent glomerulus.** Remains of the capillary tuft with a wrinkled GBM and epithelial cell are seen in the top right corner. The urinary space (US) delimited by Bowman capsule basement membrane (BBM and *arrows*) is filled with collagen as confirmed at higher magnification in the lower left **inset** (×10,000; **inset**, ×36,000). (From Nagle RB, Kohnen PW, Bulger RE, et al. Ultrastructure of human renal obsolescent glomeruli. *Lab Invest* 1969;21:419. Copyright © 1969 by the U.S. Canadian Division of the International Academy of Pathology.)

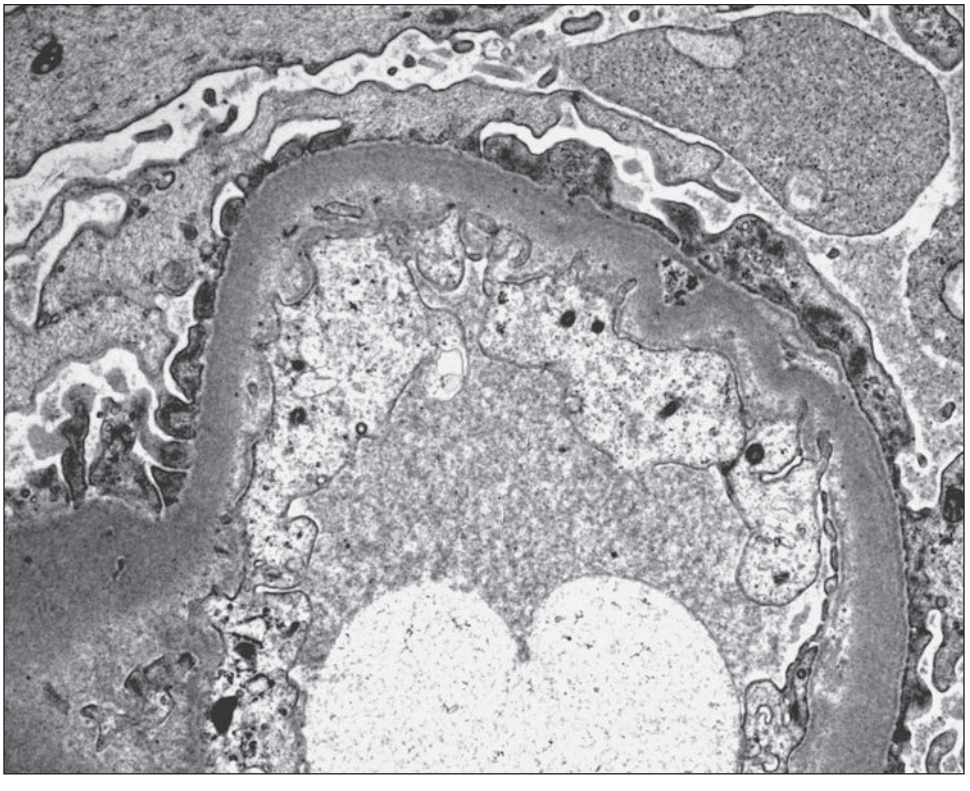

FIGURE 20.25 Electron micrograph showing mild subendothelial widening with new basement membrane formation. (Uranyl acetate and lead citrate, ×13,100.)

Ultrastructural studies (122,139) of glomeruli from patients with accelerated hypertension demonstrate dense amorphous material in the areas of fibrinoid necrosis, sometimes with fibrin tactoids. The mesangium may be intact or show areas of loosening of the matrix, sometimes with finely granular material. Loss of attachment of the GBM to the matrix denotes areas of mesangiolysis (139), which results in capillary dilation and even microaneurysm formation, an alteration that can also be seen in the other conditions associated with TMA (see Chapter 18). Red blood cell fragments, platelets, and fibrin thrombi may be found in arterioles and capillaries. Endothelial cells may be swollen or, in more severely affected areas, they may become necrotic and disappear. The widened subendothelial space contains flocculent material of variable density, sometimes with fibrin tactoids. The glomeruli with long-term changes show wrinkling of the GBM, which becomes both thicker and denser. Areas with subendothelial widening have more flocculent material, which becomes more lucent, and new basement membrane material is laid down immediately below the endothelial cell (Fig. 20.26). Ultrastructural studies have suggested that the smooth muscle cells of the media are hypertrophied (122). Furthermore, such studies have also shown the presence of cells with smooth muscle features in the intima (122).

Ultrastructural studies of vessels in severe hypertension confirm the presence of fibrin within fibrinoid necrosis, with frequent tactoids noted (122). Smooth muscle cells are necrotic or show various degenerative changes, including loss of microfibrils, vacuole formation, and increased numbers of autophagosomes. In those areas where the endothelium remains intact, one may see swelling of these cells or increased microfilaments, so-called stress fibers, at either the luminal surface or near its attachment to the basal lamina (140,141). An increase in the number of cellular organelles and myofilaments has been shown to occur in vascular endothelium when exposed to shear stress in vitro (142,143).

Etiology and Pathogenesis

Pathophysiology of Hypertension

The pathogenetic mechanisms of essential hypertension are complex, but it is recognized that sodium and fluid balance and vasomotor tone are the major determinants of blood pressure. A discussion of sodium and fluid balance is beyond the scope of this chapter. However, changes in vascular tone are related to structural changes in vessels. Thus, factors that affect vascular tone and autoregulation are described. Vascular resistance is determined by the Hagen-Poiseuille law and is directly related to the length of the vessels and the blood viscosity, as

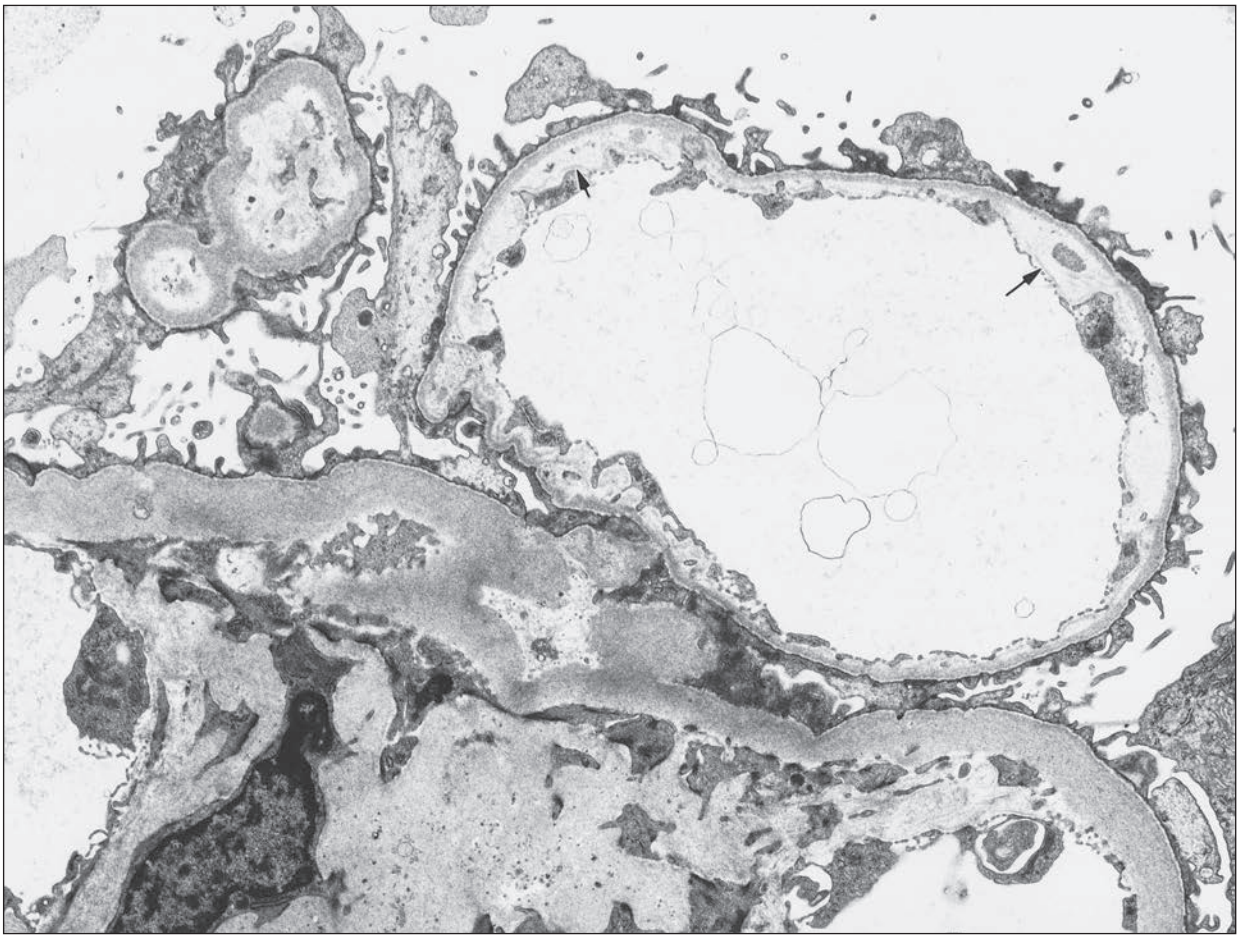

FIGURE 20.26 Electron micrograph of a portion of glomerular tuft showing a capillary loop with subendothelial widening and the new basement membrane (*arrows*) immediately subjacent to endothelial cell. The electron-lucent area between the two basement membranes contains flocculent material, most notably to the left. Occasional denser material may represent degraded fibrin-related material. (Uranyl acetate and lead citrate, ×5650.)

represented by the hematocrit level. Resistance is inversely related to the fourth power of the diameter of the blood vessels (144). Arteriolar diameter is the most powerful determinant of resistance in the short term. In the long term, other factors, such as an increased wall-to-lumen ratio, which affects lumen diameter, and rarefaction, which results in a decrease in the number of arterioles and capillaries, also become important. Many factors contribute to the vascular changes including the sympathetic nervous system (SNS), the RAS, and other hormonal regulators of vascular tone, endothelial dysfunction, uric acid, and inflammation. These are discussed briefly in turn.

The SNS is clearly involved in blood pressure regulation. Sympathetic activation occurs early in essential hypertension (145). Furthermore, the level of activation is directly related to severity of hypertension and is associated with greater cardiovascular morbidity and mortality (145). The SNS plays a role in chronic hypertension chiefly by increasing tubular sodium reabsorption, renin release, and renal vascular resistance (146). These changes shift the pressure-natriuresis curve to the right so that there is a resetting of arterial pressure up in order to reestablish sodium balance (146,147). SNS activity is increased in hypertensive patients even at an early stage of hypertensive-associated renal dysfunction and increases with reduction of glomerular filtration rate (148). A number of mutations in genes associated with sympathetic activation have been recognized in essential hypertension as well as in experimental models of hypertension (61,145). The primary role of the SNS as a mechanism in the causation of hypertension is supported by the recent development of a technique to produce renal denervation by catheter-based radiofrequency ablation of renal nerves (149). Renal denervation was associated with long-term lowering of arterial pressure in a proof of principle study in patients with hypertension that had been difficult to control (149). The role of stress and the SNS in acute hypertension is well accepted (150). Mental stress has been shown to cause a larger increase in GFR in hypertensive patients than in normotensive subjects (146) perhaps indicating greater reactivity in vessels to SNS changes. Increases in SNS activity are also important in secondary hypertension.

The RAS plays a central role in blood pressure regulation. AII is the most potent of the peptides in the system with the AT_1 receptor activating most hypertensive effects (147). AII causes renal vasoconstriction, increases the sensitivity of the tubuloglomerular feedback (TGF), and increases tubular reabsorption of sodium. AII may also have direct toxic effects on vessels and/or may be a stimulus to remodeling (see p. 867). Intrarenal AII levels are also increased in hypertension, resulting in the resetting of the pressure-natriuresis relationship. The classical view of the RAS in the kidney held that circulating AII exerted its effects on the afferent arterioles. Navar et al. (151) have studied the intratubular RAS and found abundant ATI receptors throughout the tubules of the nephron. Furthermore, intratubular concentrations of AII are higher than those of plasma, and AGT is synthesized in proximal tubule cells. Urinary AGT levels are elevated in hypertensive patients supporting the contention that this system is important in blood pressure regulation.

Endothelial dysfunction was initially defined as impaired vasodilation in response to such substances as acetylcholine or bradykinin. However, now it is recognized as a reduction in the expected vasoactive, anti-inflammatory, antiatherogenic, and anticoagulant properties of the endothelium induced by a reduction in NO bioactivity (152,153). Other factors involved in endothelial dysfunction include oxidative stress and up-regulation of inflammatory mediators. Endothelial dysfunction has been described in humans with hypertension and in families prior to the onset of hypertension (154). Some subsets of patients with hypertension do not show endothelial dysfunction (153). It is not yet clear whether endothelial dysfunction is a cause or effect in hypertension. However, its presence does signify increased risk for target organ damage.

NO is synthesized from L-arginine by a family of NO synthases and exists in two constitutive forms: endothelial and neuronal. The constitutive forms of NO are both calcium and calmodulin dependent. Release of the constitutive forms of NO produces various effects, including smooth muscle relaxation, inhibition of platelet aggregation and adhesion, and increased neurotransmission in peripheral sympathetic nerves. Larger, local doses affect both afferent and efferent arterioles. Loss or decrease in NO is one of the signs of endothelial dysfunction (152,153). Diminished NO can result from a decrease in endothelial NO synthase (eNOS) due to inhibitors or reduction in its substrate, arginine. NO synthase is inhibited by L-nitro ethyl arginine as well as by certain other substances that are normally produced endogenously (155). Long-term blockade of NO synthase brings about sustained hypertension and end-organ damage (156). RhoA, a member of the Rho family of small G proteins, plays a role in control of vascular tone (157). RhoA-Rho kinase signaling decreases eNOS synthesis by interfering with eNOS mRNA stability and possibly by stimulating arginase activity (157). Reactive oxygen species (ROS) have been associated with decreased NO mediated by an uncoupling of eNOS to a reductase function where it produces more ROS and less NO (158). Oxidative stress is defined by an imbalance between the concentration of free radicals and antioxidant defenses. During states of oxidative stress, ROS cause vasoconstriction and may be a precursor to hypertension. In the desoxycorticosterone-salt model of hypertension, Zhang et al. (159) showed that mitochondrial-related oxidative stress was associated with hypertension, which could be attenuated by inhibitors of the mitochondrial respiratory chain complex. In an in vitro model of vessels from skeletal muscle, Huang et al. (160) demonstrated that elevated shear stress could induce ROS that then diminished NO. Similarly, in human beings, markers of oxidative stress have been associated with lower levels of NO in hypertension and in chronic renal disease (152).

Hyperuricemia is also associated with increased blood pressure. Numerous epidemiologic papers including several longitudinal studies have established an associative risk between hyperuricemia and essential hypertension (161). This effect is more striking in young and middle-aged populations than in the elderly (96). Mazzali et al. (162) developed an animal model of hyperuricemia in the rat in which the hyperuricemia induced reduction in endothelial NO and stimulated renin production. Furthermore, they showed that the arteriolar lesions that developed were followed by the development of salt sensitivity that was independent of the level of serum uric acid (163). Additional studies showed that uric acid can enter vascular smooth muscle cells (VSMCs) and cause production of monocyte chemoattractant protein-1 (MCP-1) (164). Uric acid is also capable of up-regulating the production of CRP in human VSMCs (165), which leads us to a discussion of the role of inflammation in the pathogenesis of hypertension.

Numerous studies have demonstrated elevated circulating inflammatory markers in patients with hypertension (166,167). Increases in markers such as CRP or homocysteine, cytokines such as MCP-1, and adhesion molecules have been identified in patients with hypertension (167). These molecules are also elevated in prehypertensive states and indicate increased risk of developing hypertension (167). Homocysteine decreases NO bioavailability by enhancing lipid peroxidation evidenced by increased plasma levels of F_2-isoprostanes (168). These isoprostanes stimulate production of ET-1, the most powerful vasoconstrictor known. ET-1 is produced by endothelial and vascular smooth muscle cells in response to such stimuli as shear stress, hypoxia, AII, and vasopressin. ET-1 interacts with ET_A receptors to cause vascular smooth muscle contraction (169). Homocysteine and ET-1 have also been shown to have structural effects on vessels (168,170). AII also has proinflammatory actions.

Several authors in various reviews have suggested schemas that weave many of these strands into a common pathway to understand the pathogenesis of essential hypertension (161,166,171). It is possible that in the prehypertensive state repeated but intermittent episodes of vasoconstriction are initiated by a hyperactive SNS, by genetic alterations in the RAS, hyperuricemia, or a proinflammatory state. These various stimuli act through inducing oxidative stress, ET-1 release, and increased ROS to induce endothelial dysfunction and vasoconstriction. At first, the hypertension is salt resistant, and pressure natriuresis is intact. However, as arteriolar lesions develop due to effects from these various stimuli, ischemia results as well as defective autoregulation. Tubulointerstitial disease results, leading to increased sodium reabsorption. Another contributing factor to the impairment of autoregulation may be a reduction in nephron number (172,173). Arteriolar injury then reduces blood flow to glomeruli shifting the pressure-natriuresis curve and resulting in increased salt sensitivity and increased blood pressure.

The preceding discussion has introduced endothelial dysfunction and its attendant decreased NO induced by hyperuricemia, hyperhomocystinemia, and stimulated RAS as potential factors involved in the pathogenesis of hypertension. Advances in our knowledge of molecular biology have led to the uncovering of these substances and enhanced our understanding of their differing functions and interactions. In summary, the pathogenesis of hypertension remains a complex issue. Although we now can list many of the substances that play an important part in its genesis, we still do not understand all of the interrelationships among them.

Pathogenesis of Glomerular Lesions

Experimental Studies

The observations of Wilson and Byrom (174) using the 2K1C rat model, in which a renal artery from one kidney is clipped to reduce blood flow to induce hypertension, form the basis of much of our understanding of the pathologic manifestations of hypertension in the kidney. In this model, the clipped kidney is protected in the short term from hypertension, while the nonclipped kidney is exposed to the elevated systemic pressure and, as a result, manifests both glomerular and vascular injury. Changes in the nonprotected glomeruli include segmental or global necrosis with adhesions and eventual total sclerosis of the tuft. Fibrinoid necrosis is present in afferent arterioles and small renal arteries. These alterations are present in human malignant hypertension. Studies in various other experimental models of hypertension have confirmed these findings (175–177).

Many different experimental models of hypertension have been developed and are summarized by Dornas and Silva (17). Inhibition of the synthesis of NO synthase by nitro arginine methyl ester (NAME) produced vasoconstriction (156). When NO synthesis was inhibited for 2 months, blood pressure was elevated, the GFR decreased, proteinuria occurred, and glomerular injury appeared. Addition of sodium chloride to the diet exacerbated the hypertension and the glomerular injury (178). The glomerular lesions included glomerular collapse, segmental necrosis, and segmental glomerulosclerosis. Those glomeruli that showed collapse had decreased perfusion, whereas those glomeruli with either sclerosis or necrosis had been exposed to increased pressures.

Although ischemia due to vasoconstriction or luminal narrowing seems likely to be important in the collapsing form of glomerular injury in hypertension, it does not adequately explain all the alterations seen. Increased glomerular pressure may occur due to increased post-glomerular pressure, leading to increased pressure within the glomerulus itself. Early evidence that the direct transmission of increased pressure to the glomerulus could induce injury came from the studies of Byrom (179), in which removal of the clip from animals with 2K1C hypertension produced acute glomerular injury. More studies have supported the concept that increased glomerular pressure, whatever its pathogenesis, can be crucially important as a mechanism for direct glomerular injury. This increased glomerular capillary pressure results from the loss of effective afferent arteriolar constriction in the face of increased systemic pressure (i.e., loss of autoregulation) confirmed by micropuncture studies in various experimental models of hypertension (180–182). Autoregulation refers to the ability of the kidney to maintain renal blood flow and GFR in a narrow range despite wider variation in systemic blood pressure. This is accomplished chiefly through two mechanisms, namely, a myogenic reflex in the afferent arteriole and TGF (183). During impaired autoregulation, the sensitivity of TGF is reset due to changes in NO and AII. When there is sustained delivery of NaCl to the macula densa, the neuronal type of NO synthase is increased offsetting the usual vasoconstrictive effect of TGF on the afferent arteriole. These changes are found early in the course of the development of hypertension in the spontaneously hypertensive rat, the Milan hypertensive strain, and the Dahl salt-sensitive rat (reviewed in (183)). It is thought that this mechanism may be particularly important in salt-sensitive hypertension. The vasodilation resulting from the loss of autoregulation allows the transmission of higher pressure to the glomerulus, in effect an increase in glomerular capillary pressure. The role of loss of autoregulation as one mechanism for the hemodynamic alterations has been studied extensively by Griffin et al. in several different models (184–186). In the normotensive renal ablation model, the systemic pressure is slightly higher than in controls but does not attain hypertensive levels. Nevertheless, when followed for 14 to 15 weeks, these animals develop glomerular lesions that correlate to the level of their systolic pressure as well as their pulse pressure (186). The authors believe that it is the transmission of this elevated pressure to the glomerulus that results in the glomerular injury. This supposition was also

supported by the comparison of changes in autoregulation in the stroke-prone spontaneously hypertensive rat (SHRsp) and their progenitors, the stroke-resistant SHR (184). Additional evidence for the role of loss of autoregulation in glomerular injury in the setting of hypertension was provided in a comparison of four different models of hypertension (177). The two models with increased preglomerular resistance manifest less glomerular injury than the two models with decreased preglomerular resistance (177). Glomeruli throughout the cortex may be affected, with juxtamedullary glomeruli affected slightly more frequently (177). In models with vasoconstriction, the glomerular injury is much less severe and is almost restricted to juxtamedullary glomeruli (177). This finding is thought to be related to a greater loss of autoregulation in juxtamedullary as compared with superficial glomeruli in these two models.

The endothelial injury from this increased glomerular pressure may be due to shear stress, direct mechanical injury, and/or oxidative stress. The forces that bring about visceral epithelial cell injury have been studied by Kretzler et al. (187) and involve capillary loop distension with epithelial cell hypertrophy and eventual loss of attachment of the epithelial cell to the GBM. In addition, epithelial cells may show blebs and vacuoles. A possible link between mechanical strain and podocyte injury has been studied in vitro by Durvasula et al. (188). They found that increased mechanical strain was followed by increased AII production, a fivefold increase in AT_1 receptor mRNA expression, and an increase in transforming growth factor–beta (TGFβ) mRNA expression. This was also accompanied by an increase in apoptosis of the epithelial cells (188). Endothelial dysfunction may precede hypertension and is associated with decreased NO levels and an increase in AII resulting in impaired vasodilation, platelet activation, and increased vascular permeability (189,190). Microalbuminuria is a marker of endothelial dysfunction. The Ren-2 transgenic rat model of hypertension has increased tissue activity of the RAS and manifests hypertension, proteinuria, and insulin resistance (191). Effacement of foot processes is noted in glomeruli of such rats accompanied by decreased cortical expression of nephrin (191). Increased levels of 3-nitrotyrosine (3-NT), a surrogate for lipid peroxidative and oxidative stress, were present in renal cortex of Ren-2 rats. Treatment with a specific renin inhibitor restored the nephrin expression and the foot processes. Concomitant decrease in 3-NT was also found (191). In previous experiments, these investigators had shown that Nebivolol, a beta blocker that increases NO synthesis by endothelial cells, abrogated the proteinuria in Ren-2 rats accompanied by decrease in ROS/NADPH oxidase production (192).

Clinical Studies

Obsolete glomeruli show two different forms. In the first of these forms, the obsolescent glomeruli are characterized by wrinkling of the GBM with retraction of the tuft to one side of the Bowman capsule and collagenization of the Bowman space (see Fig. 20.5). This may be viewed as a form of collapse of the glomerular tuft. In the second form, the glomeruli are solidified or hyalinized; thus, an increase in matrix is often accompanied by remnants of hyalinosis lesions (Fig. 20.27). The obsolescent lesion is predominant in essential hypertension. The wrinkled glomerulus is likely the result of chronic ischemia. Kasiske (193) compared autopsy kidneys from patients with moderate to severe systemic atherosclerosis to those from patients of similar age with mild atherosclerosis with respect to the number of sclerotic glomeruli, the degree of vascular narrowing of arcuate and interlobular arteries, and the presence or absence of hypertension. He found that the extent of glomerular obsolescence depended on both the age of the patient and the degree of intrarenal vascular disease. Although hypertension correlated with the degree of glomerulosclerosis, it was not independent of the other two factors. The degree of narrowing of the renal artery correlated strongly with the extent of glomerular obsolescence, a finding supporting the idea that the sclerosis was due to ischemia, as represented by the vascular narrowing. It is well known that glomeruli may become sclerotic with increasing age, independent of increased blood pressure (110,194). Kaplan et al. (110) stated that 95% of the population aged ≤40 years should have fewer than 10% globally sclerotic glomeruli. Kappel and Olsen (194) countered that no more than 1% of glomeruli should be obsolete by age 40 but that as many 30% of glomeruli may become globally sclerotic by age 80.

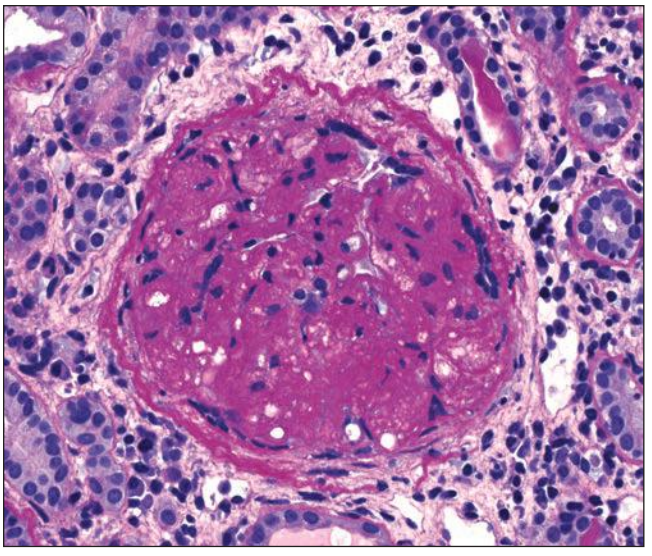

FIGURE 20.27 Solidified glomerulus extending to the Bowman capsule with increased mesangium, sclerosis of capillary loops, and occasional foam cells. (PAS, ×400.)

Hypoxia has been heavily implicated in the pathogenesis of interstitial fibrosis (126,195) (see p. 870). Recent studies suggest possible mechanisms for a role in glomerulosclerosis as well. Neusser et al. (196) examined gene expression data in microdissected glomeruli from patients with hypertensive nephropathy and found an increased expression in several target genes of hypoxia-inducible factor (HIF). Furthermore, HIF-1α was identified in podocyte nuclei as a marker of transcriptional activation.

The solidified glomerulus is the alteration that Bohle described in DBN (113,114), a form associated with a more rapid course to ESRD. Marcantoni et al. (74) studied 62 hypertensive patients (19 African Americans and 43 Caucasians) with regard to the types of glomerular injury present in their renal biopsies. They found that 25% of glomeruli in the African American patients were of the solidified form as compared to 8% in Caucasians. A subgroup of patients also had segmental sclerosis, also seen more frequently in African Americans. In

addition to the segmental lesions, the African Americans in this subgroup had 38% solidified glomeruli. The higher prevalence of the solidified glomerulus in African Americans with hypertension was first associated with the more common occurrence of polymorphisms in the nonmuscle myosin heavy chain 9 (*MYH9*) gene in African Americans (69). Polymorphisms in *MYH9* have been linked to FSGS and other renal diseases in both African Americans and Caucasians (69). More recently, this association has been found to be due to missense mutations in apolipoprotein-1, which is closely linked to MYH9 (76). Loss of autoregulation has also been suggested as a potential cause of the solidified glomerulus (111,116,197). Hill et al. (116) studied the relationship between arteriolar diameter and glomerular appearance in hypertensive individuals and found that no correlation was present in normal appearing glomeruli. However, hypertrophied glomeruli that were solidified or showed FSGS-like lesions demonstrated a correlation between arteriolar diameter and glomerular size. They suggested that this finding supported loss of autoregulation (116). Another factor in the pathogenesis of these lesions may be the presence of reduced numbers of glomeruli in some patients with hypertension (8,172,173). Brenner and Chertow (172) suggested that early gestational age or fetal growth retardation might affect nephrogenesis so that a reduced number of nephrons occur with consequent hyperfiltration of those that are present. Keller et al. (173) found a reduced number of nephrons at autopsy in patients with hypertension. Podocyte loss may also contribute to this lesion. Wang et al. (118) have demonstrated decreased podocyte number in glomeruli from hypertensive patients as compared to glomeruli from donor kidney biopsies. Podocyte loss has been recognized as a risk factor in the development of FSGS as well as other glomerular lesions (198).

Pathogenesis of Small Vessel Changes

Considerable examination of the pathogenesis of the different vascular changes seen in human hypertension has been undertaken over the years. These changes include hyaline arteriolosclerosis, intimal thickening, and medial thickening. Each of the major alterations is discussed in turn.

Hyaline Arteriolosclerosis (Arteriolar Hyalinosis)

Hyaline arteriolosclerosis is an alteration seen in patients with benign hypertension, in the aged, in patients with diabetes mellitus, and in patients with a variety of glomerular diseases. Thus, it is not specific for hypertension. Moritz and Oldt (199) examined small arteries and arterioles defined by an external diameter less than 500 μm at autopsy from 100 patients with hypertension and compared them with 100 control cases. These researchers found that hyaline arteriolosclerosis increased with age in various organs. A clear relationship between hyaline arteriolosclerosis and hypertension was seen only in the kidney. Tracy et al. (200) confirmed that renal hyaline arteriolosclerosis correlated with blood pressure.

Many investigators used immunofluorescence techniques to identify the various materials within the hyaline lesions (135,200). C3 has always been identified, often in the company of IgM. Gamble (135) found C3 and its fragments only without other complement components or immunoglobulins. Tracy et al. (200) also found inactive C3b in hyaline lesions. In addition, these investigators found cholesterol, phospholipid, triglycerides, tryptophan, tyrosine, and cysteine, sometimes accompanied by small amounts of fibrin. This accumulation was thought to be a natural consequence of aging, but it could be accelerated by either hypertension or diabetes mellitus. In such cases, increased vascular permeability could account for the acceleration of lesion formation. Williams et al. (201) showed that AII can increase vascular permeability independent of its pressor activity in the setting of hypertension. Hill et al. (117) demonstrated in the aging kidney that vessels with nonobstructive hyaline deposits were dilated and the muscular walls were thinned even in the absence of hypertension. The glomeruli associated with these vessels were either solidified or showed FSGS-like changes. They ascribed the glomerular changes to the consequences of loss of autoregulation. These investigators found similar although more frequent such changes in a cohort of hypertensive patients (116). They supported their suggestion of the role of loss of autoregulation by citation of evidence for such alterations in experimental models of hypertension. Recently Bockmeyer et al. (202,203) have examined the phenotype of the VSMCs in the kidneys with benign nephrosclerosis from hypertensive patients and found that the markers of contractility in these cells are decreased in the vessels with arteriolar hyaline as the cells change from a contractile to a secretory phenotype. This change in phenotype supports the idea of a loss of contractility in these cells that would certainly impair autoregulation.

Intimal Arterial Thickening

The observation of intimal thickening in hypertension is well established. Hypertensive nephrosclerosis is often defined by the presence of intimal thickening or fibroplasia. Tracy et al. (204) related nephrosclerosis to arterial fibroplasia, blood pressure, and age and compared different populations in New Orleans, Japan, and Guatemala. They found that all patients had at least some arterial fibroplasia. Additional studies have shown that intimal thickening of renal interlobular arteries increases with age and is accompanied by increased blood pressure (205). However, its pathogenesis has not been defined.

The relationship between hypertension and atherosclerosis is well accepted (206). Oxidative stress plays a central role in the pathophysiology of both atherosclerosis and hypertension (152,207,208). Oxidative stress may be defined by increased production of ROS or decreased scavenging or metabolism of ROS. Shear stress and stretch may induce increased ROS via NADPH oxidase or reduced eNOS and thus decrease of NO (208). Some of the possible mechanisms for decreased synthesis include deficiency in the precursor, L-arginine, changes in the L-arginine transporter, production of endogenous NO synthase inhibitors such as asymmetric dimethylarginine (ADMA), cofactor deficiency, decreased eNOS gene, decreased half-life of eNOS mRNA, changes in Gi proteins, changes in calcium-independent pathways of eNOS activation, and changes in the interactions between eNOS and caveolin-90 (209). Shear stress has been shown to increase gene expression of protein arginine methyltransferase (PRMT-1), ADMA synthase, resulting in increased levels of ADMA (210). Increased NO degradation or inactivation occurs secondary to oxidative stress through interactions with superoxide ions mediated chiefly through increased NADPH and xanthine oxidase (208,209). However, we still do not understand the reasons for the increased intimal thickness, which is most pronounced where shear stress varies over time and is of lower magnitude.

Vascular Remodeling

The presence of increased wall thickening is an old observation in arteries from patients with hypertension. The reduced diameter of the lumen was thought to be the structural equivalent of increased peripheral resistance. An important measurement used to document this alteration is an increase in the wall-to-lumen ratio. Short (211) dilated and fixed by perfusion the mesenteric arteries of patients with hypertension and controls at autopsy. He then compared the wall-to-lumen ratio of arterioles in deciles by size and found that this ratio was higher for hypertensive patients for all but the smallest 20% of arterioles. Short also found that the cross-sectional area was not increased. Christensen (212) compared the mesenteric vessels of SHR and WKY rats. Multiple regression analysis showed that pulse pressure was the main factor responsible for change in the wall-to-lumen ratio. These changes in the vessel wall now called remodeling were first defined by Baumbach and Heistad (213) as a rearrangement of the existing cells around a smaller lumen. Two types of remodeling occur. Eutrophic remodeling is characterized by reduction in the outer diameter and lumen, but the media cross-section does not increase due to the rearrangement of the cells themselves (214). In contrast, hypertrophic remodeling is manifest by encroachment of the lumen due to the increased size of the muscle cells with no change in the external diameter (214). Both forms are seen in hypertension with the eutrophic form predominating in essential hypertension at least in early stages. Hypertrophic remodeling is seen more frequently in the secondary forms of hypertension and may represent a maladaptive compensation in response to chronic blood pressure elevation (215).

These vascular structural changes are induced by endothelial dysfunction and mechanical strain (216) secondary to a complex process that involves extracellular matrix components, adhesion molecules, apoptosis, and both proinflammatory and profibrotic cytokines (128,217). AII is critical in many of these processes. AII induces vascular smooth muscle hypertrophy via the AT_1 receptor and activation of the Rho kinase pathway (217,218) enhancing hypertrophic remodeling. However, AII also increases apoptosis and is profibrotic via TGFβ (217). AII is proinflammatory causing increased cytokines and increases in ROS through activation of NADPH oxidase (see Fig. 20.27). Low-grade chronic inflammation is now recognized as an important player in these changes (217). A detailed discussion of the role of AII in vascular remodeling is provided by Marchesi et al. (217). Mechanical strain may directly affect the VSMCs by mechanotransduction involving extracellular matrix-integrin-cytoskeleton links that result in cytoskeletal alterations as well as potential cell signaling that affects the ability of the VSMC to respond to the strain (219,220). VSMCs can undergo phenotypic switching from the baseline contractile phenotype to a secretory phenotype with intermediate forms (203,220). This switching is under the control of both platelet-derived growth factor and TGFβ (220) and may play a role in eutrophic remodeling. Both forms of remodeling may occur in the same patient or experimental animal at the same time, and these changes may increase with longer exposures to hypertension (128).

Remodeling of the smooth muscle cells of the vessels is affected by an increase in the intercellular matrix in vessels of all sizes. O'Callaghan and Williams (221) showed a link between chronic cyclical strain and increased production of extracellular matrix by VSMCs grown in culture. This was mediated by increased TGFβ. Connective tissue growth factor (CTGF) is also increased in resistance vessels from patients with hypertension accompanied by increases in collagens III and IV (222). Such changes result in increased stiffening and decreased compliance in all vessels and are associated with increased cardiovascular morbidity and mortality (223). Yasmin et al. (224) studied arterial stiffness using an augmentation index and pulse wave velocity in the young adult offspring of hypertensive and nonhypertensive families. They found that the augmentation index was increased in the offspring of hypertensive families suggesting that patients likely to develop hypertension might have changes in arterial compliance prior to the onset of increased blood pressure. In the resistance vessels, these changes in the extracellular matrix further increase the thickness of the media in hypertrophic remodeling and contribute to the reorganization of the vessel wall in eutrophic remodeling (128,223). MMP-2 is increased in the vessel walls throughout the course of 2K1C hypertension model from the early to late phases and is associated with increased extracellular matrix deposition and oxidative stress (225). Plasma levels of MMP-2, MMP-9, and tissue inhibitor of MP were elevated in hypertensive patients with cardiac remodeling as compared to controls suggesting that these molecules are also important for human hypertension vascular remodeling (108).

As discussed above in the "Pathophysiology of Hypertension" section, increased levels of uric acid in the serum may also play a primary role in essential hypertension (171). In a rat model of hyperuricemia, Mazzali et al. (162) showed that the elevated serum uric acid produced hypertension that was associated with arteriolopathy of the afferent arteriole. Micropuncture studies of these rats showed that the hyperuricemia was associated with vasoconstriction and a decrease in renal plasma flow (226). Kang et al. (165) demonstrated that uric acid up-regulated CRP mRNA expression in human VSMCs in culture. Furthermore, uric acid increased proliferation and induced migration of these cells. Treatment of these cells with an anti-CRP antibody reversed the effects suggesting that they had been mediated by CRP. Uric acid may be another factor that causes vascular remodeling in the setting of increased oxidative stress.

Aldosterone is also emerging as an important player in the pathogenesis of hypertension and in the structural changes in vessel walls (227–229). Aldosterone induces both hypertrophy and pericellular fibrosis in VSMCs (229). Most of the hypertension associated with aldosterone represents secondary forms and is discussed in that section of the chapter. However, aldosterone may have effects on the vasculature in essential hypertension as well particularly in states of relative aldosterone excess even if the serum levels are within the normal limits (228). Aldosterone can act synergistically with AII, in part by up-regulating the AT_1 receptor, to increase ROS and all of the effects that follow from that interaction (228). Tsukoba hypertensive mice are double transgenic mice that are produced by crossing human renin overexpressing mice with human AGT overexpressing mice (227). They have elevated AII, are hypertensive, show end-organ damage, and have elevated aldosterone in their urine. Treatment of these mice with spironolactone, an aldosterone blocker, reduced intimal thickening and vascular remodeling of the aorta despite maintenance of increased AII levels and hypertension (227). These findings support the synergistic effects of aldosterone with AII.

Remodeling can be reversed with therapy as shown in both experimental models and in human beings (215,222,230–235). Notoya et al. (233) administered an angiotensin-converting enzyme (ACE) inhibitor to SHR and found a reversal of vascular remodeling. Ledingham and Laverty performed a series of studies in the New Zealand genetically hypertensive rat. They found that an ACE inhibitor prevented remodeling as did two different angiotensin receptor antagonists (231,232). Rehman et al. administered an angiotensin type 2 receptor alone or in combination with losartan, an angiotensin type 1 receptor antagonist (AT_1RA) to stroke-prone hypertensive rats, and examined the heart, aorta, and kidney. They found reduced expression of two genes associated with cardiac hypertrophy as well as decreased ROS production by the aorta. Furthermore, monocyte/macrophage and T-cell lymphocytic infiltration were reduced in the kidney. They concluded that an angiotensin type 2 receptor agonist alone or in combination with losartan was able to alter vascular remodeling. Schiffrin et al. (235) contrasted the ability of losartan to that of atenolol, a β-blocker, to reverse remodeling in resistance arteries in subcutaneous arteries of human beings. They found that the AT_1RA reduced the ratio of media width to lumen diameter following 1 year of treatment but that atenolol did not have such an effect. In a similar system, Gomez-Garre et al. (222) showed that hypertensive patients receiving losartan had a decreased media-to-lumen ratio, while the patients receiving amlodipine had a further increase in that ratio. This result indicated an increase in vascular remodeling in the patients receiving the β-blocker. The patients receiving losartan also had decreased amounts of CTGF and collagen IV relative to those receiving a β-blocker despite similar control of blood pressure (222). Schiffrin (234) has reviewed the various studies of the effect of different antihypertensive agents on the structure of small arteries in patients. This review showed that the ACE inhibitors, AT_1RAs, and calcium channel blockers reversed remodeling. However, the β-blockers did not (234). A more recent review of 15 studies involving a total of 344 hypertensive patients confirmed the findings listed above and added hydrochlorothiazide to the list of drugs not effective in reducing media/lumen ratio (215). Possible explanations for these differences include lack of vasodilation seen with at least some β-blockers and a possible decrease in arterial stiffness with the various AII inhibitors (215). This reduction in stiffness may be due to reduction in collagen as seen in the work of Gomez-Garre et al. (222).

Summary

The mechanism for vascular remodeling is complex with many of the signaling pathways now delineated. It seems likely that different factors are important depending on the type of hypertension and the underlying genetic abnormalities. However, a broad outline may be seen in the accompanying figure (Fig. 20.28). Several different inciting factors may be involved in the vascular injury including prolonged shear stress, AII, aldosterone, and uric acid. Shear stress is transduced by integrins as well as other matrix components to cause release of several factors that may promote remodeling

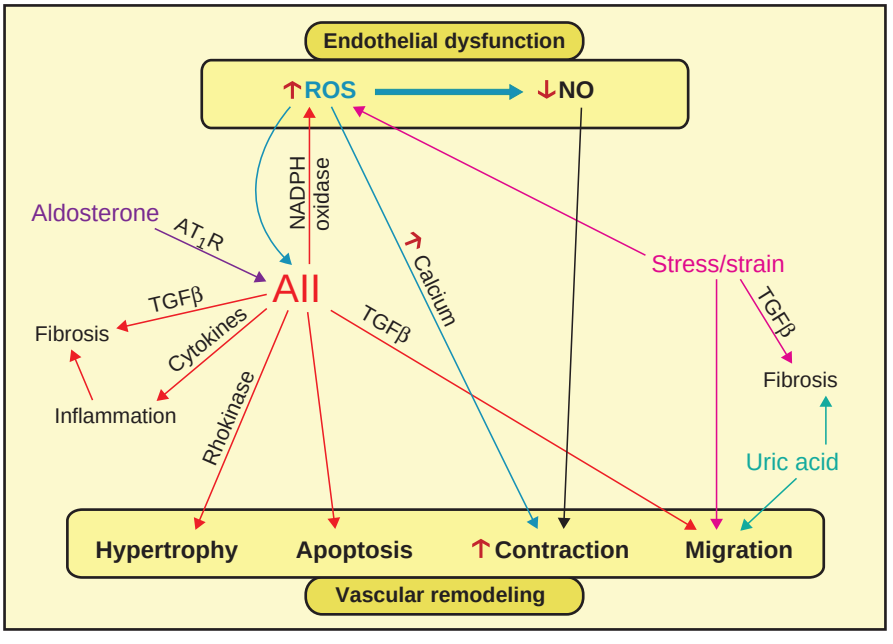

FIGURE 20.28 This figure shows some of the factors that are involved in vascular remodeling, including the effects of endothelial dysfunction. The major inciting factors are angiotensin II (AII), aldosterone, stress/strain, and uric acid. AII is central and affects all four components of vascular remodeling directly (apoptosis, hypertrophy mediated by Rho kinase, and migration mediated by transforming growth factor beta (TGFβ) or indirectly (contraction by increasing reactive oxygen species [ROS] in the endothelium). Aldosterone increases AII through the angiotensin 1 receptor (AT_1R). Stress/strain increases endothelial ROS and thus increases contraction as well as affecting myocyte migration. Stress/strain also contributes to fibrosis and thus wall stiffening mediated by TGFβ. Finally, uric acid affects cell migration and also increases fibrosis. All also contributes to intercellular fibrosis mediated by TGFβ and by inducing inflammation via cytokines.

as well as to increase overall vascular stiffening. AII increases ROS mediated by NADPH oxidase resulting in decreased NO that increases vasoconstriction and reducing vasodilation. AII also produces muscular hypertrophy mediated through the Rho kinases, releases cytokines leading to inflammation, and increases TGFβ leading to intercellular fibrosis. The increase in ROS contributes further to inflammation and mediated by ERK and MAPK among others, the change from a contractile to secretory phenotype occurs. This results in both smooth muscle hypertrophy and migration. Uric acid contributes to enhancing smooth muscle migration. Aldosterone acts synergistically with AII by up-regulation of the angiotensin II type 1 receptor (AT_1R). A review of many of these pathways may be found in Lacolley et al. (220).

Pathogenesis of Interstitial Disease

Considerable interest exists in the relationship between changes in the interstitium and in the remainder of the kidney in hypertension as well as in other renal disease. The changes are thought to represent a final common pathway to end-stage disease. Bohle et al. (236) were the first to recognize the role of loss of interstitial capillaries, so-called capillary rarefaction, in progressive tubular atrophy and interstitial fibrosis. Fine et al. (126) put forward the "chronic hypoxia hypothesis" as an explanation for the progression of interstitial fibrosis in 1998 and then elaborated further a decade later (195). In brief, they proposed that downstream damage to the tubulointerstitial network of capillaries from any of a number of different insults resulted in tissue hypoxia and a fibrotic response in tubulointerstitial cells. In the setting of hypertension, such injury might be secondary to vasoconstriction, resulting in lowered tissue oxygenation (111). The possible contribution of elevated AII was delineated in the model of renal ablation in which reduced capillary blood flow and tissue oxygenation were demonstrated prior to the appearance of visible structural damage (237). That hypoxia may precede the actual loss of capillaries was also demonstrated by Matsumoto et al. (238). The response to hypoxia by the proximal tubular epithelial cells is orchestrated by the family of HIFs with resulting epithelial-mesenchymal transformation, increased matrix formation, reduced matrix degradation, and with prolonged hypoxia, apoptosis of tubular cells (195). Macrophages are also recruited to the interstitium. The importance of these processes has been demonstrated in human renal disease as well. Eardley et al. (239) examined renal biopsies in 110 patients with chronic renal disease (37 with hypertensive/ischemic nephropathy) and found an inverse correlation between monocyte number in the biopsy and capillary density. A similar inverse correlation was present between urinary MCP-1 and capillary density. In the subgroup with hypertensive nephropathy, capillary density decreased with increasing chronic damage index. A similar trend was seen with macrophage infiltration and chronic damage (239). Treatment with rosuvastatin in an AII-induced model of hypertension and renal injury reduced renal injury and mortality (240). Administration of the drug reduced both neutrophil and monocyte infiltration in a dose-dependent fashion and were accompanied by reduction in renal injury.

Differential Diagnosis

Several conditions resemble the pathologic changes seen in hypertension. These include aging, cocaine-associated nephropathy, TMA, and antiphospholipid antibody syndrome (APS). Hypertension may be a complication of other renal diseases as renal parenchyma becomes scarred during the progression of renal disease. These are discussed in turn. The secondary forms of hypertension may produce changes that are indistinguishable from essential hypertension. The pathology of these forms is discussed later in the chapter.

Aging produces many of the same changes seen with hypertension in both experimental models and in humans (110,117,204,241–243). These include glomerular obsolescence of the wrinkled capillary wall type, interstitial fibrosis, tubular atrophy, intimal thickening, and hyaline arteriolosclerosis (110,117,204,243,244). However, the solidified form of obsolescence is not expected due to aging. In addition, although some remodeling of vessel walls may occur with increased age, widespread medial thickening is unusual. Finally, reduplication of elastic lamina is not usually seen in older individuals in the absence of hypertension. The similarities are likely due, in part, to the common mechanism of ischemia (242–244). However, molecular mechanisms including control of nephron number, senescence marker protein 30, or decreased expression of *klotho* have also been associated with accelerated renal aging (244,245).

Cocaine abuse has been associated with many different forms of renal damage. Malignant hypertension may occur secondary to vasoconstriction and may show TMA in the kidney (88). A study in HIV-infected individuals showed hypertensive-related changes in the kidneys in the absence of diabetes or documented hypertension (246). These changes included hyaline arteriolosclerosis and arterial intimal thickening and fibrosis.

TMA is characterized by the presence of fibrin platelet thrombi with schistocytes contained within arterioles and glomerular capillaries. Subendothelial widening with double contours on silver stain is frequently seen. TMA is a common pathologic finding in several different disorders including malignant hypertension, hemolytic-uremic syndrome, thrombotic thrombocytopenic purpura, scleroderma, and with the administration of a number of drugs such as calcineurin inhibitors and various chemotherapeutic agents. It is not possible to distinguish among these diagnoses without clinical information. These disorders are discussed in Chapter 18.

APS is defined by the presence of antiphospholipid antibodies and/or lupus anticoagulant–associated arterial or venous thrombosis and spontaneous miscarriages (247,248). The patients frequently have severe hypertension as well. It may occur as a primary lesion or may complicate autoimmune disease, in particular systemic lupus erythematosus (249,250). Nochy et al. (247) examined the changes in the kidney of 16 patients with primary APS. They found TMA in 31%, fibrous intimal hyperplasia in 75%, fibrous and fibrocellular vascular occlusions in 68%, and focal cortical atrophy in 61% of the biopsies. The fibrous intimal hyperplasia frequently showed a cellular proliferation with actin-positive cells. They found the involvement of larger vessels, the increased cellularity of the intimal hyperplasia, and the focal cortical atrophy to be useful features allowing differentiation from TMA in general. In addition, the medial walls did not show the changes typically associated with essential hypertension.

CKD is commonly accompanied by hypertension (251,252). Fifteen to eighty percent of patients with chronic glomerular disease without renal failure had hypertension (251). In a study of patients with ESRD, the prevalence of

hypertension was 90% (252). The lesions are identical to those described above for essential hypertension. The differentiation between primary hypertension and hypertension complicating a parenchymal renal disease is made by the presence of a primary glomerular or tubulointerstitial disease preceding the onset of hypertension. In addition, if collapsed and wrinkled glomeruli predominate, then the likelihood increases that hypertensive vascular injury has contributed to the changes seen, whereas if the glomeruli show the solidified form, then an end-stage kidney disorder is more likely to have resulted from some other glomerular disease such as chronic glomerulonephritis in which enlargement or expansion of the glomerulus may precede the sclerosing process (see Chapter 28).

Clinical Course, Prognosis, Therapy, and Clinicopathologic Correlation

Clinical Course

The natural history of hypertension has changed and continues to evolve as new antihypertensive drugs are developed. Review of older studies, before effective therapy protocols were developed, provides an interesting backdrop to a consideration of the current progression of the disease. One of the best of these studies is that of Perera (253) who studied 500 untreated hypertensive patients with diastolic pressures greater than 90 mm Hg. He found that 50% of patients suffered from congestive heart failure, 16% had angina, and 12% had strokes. These complications led to shortening of life by 15 to 20 years. Seven percent of patients, mostly men, progressed to the malignant phase of hypertension. Effective therapy has resulted in a lower mortality rate from the hypertension itself and has reduced the incidence of complications. However, in the age group between 35 and 64 years, hypertension continues to increase risk for coronary disease, 2 and 2.2 times for men and women, respectively, for stroke, 3.8 and 2.6 times, for peripheral artery disease, 2 and 3.7 times, and for heart failure, 4 and 3 times (81). Risk is also increased in patients aged older than 64 with relative risks ranging between 1.6 and 2.3 for the various cardiovascular complications (81). Factors that increase the risk of progression to ESRD for a hypertensive patient include lack of treatment, diastolic blood pressure (greater than 87 mm Hg), male sex, and black race (254).

Hypertension is the second most common cause of ESRD in the United States accounting for one third of the incidence of ESRD and for approximately 125,000 patients who receive dialysis (7). However, the true incidence of primary hypertension resulting in ESRD is difficult to determine. The Multiple Risk Factor Intervention Trial showed that an increase in either systolic or diastolic blood pressure one standard deviation above the lowest group produced a 1.7-fold increased relative risk of developing ESRD despite exclusion of patients with CKD from the study (255). Hsu et al. (256) showed that the adjusted relative risk of developing ESRD is 1.62 (CI 1.27 to 2.07) for blood pressures of 120 to 129/80 mm Hg compared to patients with blood pressure less than 120/80 mm Hg. As levels of blood pressure become more elevated, the adjusted relative risk of ESRD also increases to 2.59 at blood pressures of 140 to 159/90 to 99 mm Hg and to 3.88 for blood pressures of 180 to 209/110 to 119 mm Hg. At times, a clinician evaluates a patient, discovers high blood pressure, and then finds ESRD. Renal biopsy is usually unrewarding in these late circumstances, although in a small proportion of cases, a primary glomerular disease may be found. The characteristic pathologic manifestations of hypertensive nephrosclerosis, however, are nonspecific, especially in the context of ESRD. Furthermore, it is not always possible to distinguish between primary and secondary hypertension on a renal biopsy. It is more difficult to determine the contribution of hypertension to the prevalence and incidence of predialysis CKD due to a lack of classification by underlying etiology of CKD (7). Nonetheless, hypertension is more frequent in any patient with any stage of CKD and is present in more than half of the patients with stage III CKD and above (7).

Before antihypertensive therapy was available, nearly 70% of patients died of renal failure within months of the onset of the malignant phase of hypertension (121). Lip et al. (87) in their study in Birmingham, England, showed no decline in the incidence of malignant hypertension over a 20-year period ending in 1994. The stability of incidence of malignant hypertension at 1 to 2 new cases per year per 100,000 population has been extended over 40 years (86). Demographics have also remained similar except that Van den Born et al. (92) report a higher incidence in blacks compared to whites with higher rate of complications as well. These differences are attributed to disparities in blood pressure control, medication adherence, and social status. Clinical presentation has differed since 1977 with lower serum creatinine, proteinuria, less severe retinopathy, as well as decreased left ventricular hypertrophy (86). Survival has improved remarkably from 1% at 5 years prior to the availability of antihypertensive medications to 66% at 5 years since 1977 (86). A separate cohort studied between 1974 and 2007 showed 5-year survival of 84% with 10-year survival of 72% (90). Renal function had stabilized in most of the survivors. Prognosis depended primarily on the degree of proteinuria at presentation. Effective therapy returns the patient to the typical course for hypertension, but the severe levels of hypertension can recur and put the patient at risk for cerebral hemorrhage or myocardial infarction (257). Other complications include chronic renal failure (31.7%), heart failure, hypertensive cardiomyopathy, and angina (87).

Prognosis

The factors that determine prognosis are those that affect the development of the complications of hypertension. The risks for cardiovascular disease have been outlined above. I will limit this discussion to the factors that determine the likelihood of developing renal disease and its progression in association with hypertension. Hanratty et al. (258) studied the risk factors for incident CKD in hypertensive patients and found that diabetes was the strongest predictor. They also found that each 10 mm Hg increase in baseline or time-varying systolic blood pressure led to a 6% increase in the risk of incident CKD (258). Approximately 30,000 hypertensive patients with previous renal insufficiency develop ESRD (7). In a study of more than 16,000 patients, Peralta et al. (259) showed that increased systolic blood pressure accounted for most of the risk for progression from stage 3 CKD to ESRD. Additional factors that predispose the hypertensive patient to renal failure are increasing age, poor serum glucose control in diabetic patients, metabolic syndrome, level of systolic blood pressure, nondipping of blood pressure at night, black race, and elevated uric acid and triglycerides (259–263). Effective therapy reduces the likelihood of progression to renal insufficiency (264) and may even result in improvement in renal function in some patients

(265). In this study, improvement in renal function was noted in only 3% of participants and was seen in those with low urinary protein at baseline in the group with stricter control of blood pressure. The authors caution that validation of these results is needed in other studies.

The relationship between genetic variations and the presence of renal insufficiency is being studied in hypertensive patients. Such studies have been reviewed (266). A few representative studies are presented. Mallamaci et al. (267) showed an association between the presence of the D allele in the *ACE* gene and biopsy-proven hypertensive renal disease. In a more elaborate study, Fabris et al. (268) examined polymorphisms in the renin-angiotensin-aldosterone system due to its central role in the pathogenesis of hypertension in hypertensive patients with and without renal failure. They examined the insertion/deletion of angiotensin-converting enzyme (*ACE* I/D), *AGT* M235T, AT_1R A1166C, and aldosterone synthase (*CYP11B2*)-344C/T polymorphisms. They found that each of the genetic polymorphisms examined was associated with renal failure. The associations *AGT* TT-*AT1R* AC and *CYP11B2* CC-*ACE* DD were present more frequently in hypertensive patients with renal insufficiency. On the other hand, patients with *AGT* MM-*AT1R* AA and *AGT* MM-*AT1R* AA-*CYP11B2* TT or TC combinations showed a reduced risk for renal failure (268). Linkage studies have identified some potential susceptibility loci for renal damage in hypertension, but no specific genes have been identified (266). GWAS are also in early phases (266). The association of two risk alleles in *APOL1* with renal disease, notably hypertensive renal disease and FSGS, has been found in African Americans (269). The odds ratio is 7.6 for the association between these two sequence variants in *APOL1* and hypertensive attributed end-stage kidney disease in African Americans (269). A large population-based study demonstrated that the presence of both risk alleles explained most of the ethnic disparity for the increased incidence of hypertensive renal disease among African Americans (270). The mechanism for the increased risk is not yet understood.

Therapy

Numerous drugs are available to treat hypertension. They fall into different classes of action. Those currently in use include diuretics, β-adrenergic blockers, ACE inhibitors, calcium antagonists, AII receptor antagonists, adrenergic inhibitors, and direct smooth muscle vasodilators. The value of lowering blood pressure is clear with regard to lowering risk of cardiovascular complications such as stroke to slowing the onset and later progression of hypertensive nephropathy (271).

Depending on the severity of the hypertension, patients are started with lifestyle changes such as smoking cessation, weight reduction, decreased alcohol consumption, decreased salt intake, increased physical activity, and increased fruit and vegetable consumption (271). However, in the long term, such lifestyle changes are unlikely to be successful alone. Stepped-care therapy is now employed in which optimal dosing with a first-line agent is established bolstered by additional drugs added until optimal blood pressure levels are obtained (271). The drug therapy is tailored to the individual patient's needs (272). Some common combinations might include a thiazide diuretic and either an ACE inhibitor or an angiotensin receptor antagonist, a calcium antagonist with a thiazide diuretic, or a beta blocker with a calcium antagonist of the dihydropyridine type (271). Further discussion including a decision tree and other factors to be considered may be found in Mancia et al. (271).

The original hope was that as we developed an understanding of the molecular mechanisms underlying essential hypertension, we would also be able to tailor therapy on an individual basis. However, to date, this dream has not become a reality due to the complexity of the regulation of blood pressure. Nonetheless, new directions are being pursued. A role for inflammation in the pathogenesis of hypertension is now recognized mediated at least in part by AII (273). Many of the drugs already in use have anti-inflammatory actions. Statins may have anti-inflammatory as well as cholesterol-lowering effects. Nonsteroidal anti-inflammatory drugs clearly reduce inflammation; however, blood pressure may increase with this class of agents (273). Other agents such as peroxisome proliferator–activated receptor agonists have been shown in rats to have anti-inflammatory actions including repairing vascular structure but have not been used in clinical trials for blood pressure control (273). The SNS has an accepted role in blood pressure control. Two new approaches have been described and may be used in resistant hypertension. These include renal denervation and carotid baroreceptor stimulation (145,274).

Two more speculative approaches to hypertension therapeutics are also being considered. The first of these is the development of a vaccine (274). A phase 2 trial of a vaccine against AII has been reported, but the true efficacy of this approach has yet to be demonstrated (274). MicroRNAs are transcriptional regulators that are involved in numerous biologic processes likely including hypertension. Their potential role in hypertension as well as hypothetical therapeutic targets is discussed by Batkai and Thum (275).

Clinicopathologic Correlation

Many clinical factors have been found to correlate with progression of renal as well as cardiovascular complications of hypertension. One factor that links all of these complications is the acceleration of atherosclerosis by hypertension. Several lines of evidence support this link. Patients with hypertension invariably have some degree of atherosclerosis. Blood pressure is an independent risk factor for cardiovascular disease (80). Furthermore, effective therapy for hypertension has been associated with reduced cardiovascular disease as well as other end-organ damage (80). Systolic blood pressure, in particular, has been associated with increased atherogenesis and with decreased large vessel compliance (276). Arterial stiffness also correlates with target organ damage (277). Endothelial dysfunction is an important marker of both atherosclerosis and hypertension (152,278). Endothelial dysfunction can be measured in human beings by noting the degree of vasodilation in the forearm vasculature in response to acetylcholine (152,278). Zoccali et al. (278) found that such endothelial dysfunction correlated with glomerular filtration rate in 500 patients with early, untreated essential hypertension with serum creatinine ≤1.5 mg/dL independent of blood pressure supporting its use as an indicator of renal injury. Rodriguez-Porcel et al. (279) studied the effects of hypercholesterolemia (as a model for atherosclerosis), renovascular hypertension, or both in pigs. They found that endothelial dysfunction as measured by changes in renal perfusion pressure in response to acetylcholine or nitroprusside was worst in the pigs with both hypertension and hypercholesterolemia but was depressed in the other two

experimental groups as well. These changes were paralleled by a decrease in oxygen radical scavengers suggesting that hypertension and hypercholesterolemia can damage endothelial cells but that the two together can act synergistically to cause greater damage (279).

Clinical factors predicting the presence of renal injury in the setting of hypertension include microalbuminuria and increased serum creatinine at presentation. Microalbuminuria is associated with increased risk for cardiovascular disease (7). Recent studies indicate that microalbuminuria may not be a good marker for progression of renal disease in clinical studies due to the difficulty in accurate measurement (280,281). Progression to macroalbuminuria may signal the onset of glomerular disease due to either endothelial injury with dysfunction or glomerular hyperfiltration (7). Pathologically one may find the solidified type of glomeruli. Vikse et al. (282) performed a retrospective study with a 13-year follow-up on 102 patients who had a renal biopsy that confirmed the diagnosis of hypertensive nephrosclerosis. Proteinuria (greater than 1 g/24 hours) and doubling of serum creatinine had a significant risk of progression to ESRD over the 13 years of follow-up. Each time the serum creatinine doubled, the relative risk of progressing to ESRD increased by 2.6 times. Likewise, each time the proteinuria increased by 1 g/24 hours, the relative risk of ESRD increased by 1.4 times. Patients with increased systolic blood pressure were at increased risk for ESRD during the first 3 years of the follow-up. In severe levels of hypertension, the serum creatinine level at the time of diagnosis has been a good indication of the outcome with regard to chronic renal function. Cystatin C is a new addition to the biomarkers of renal function that shows early promise as an indicator of hypertension-associated renal dysfunction (7). Additional techniques that may prove useful as a predictor of renal function include measurement of the media/lumen ratio of small resistance arteries in subcutaneous fat biopsies (283) and plethysmography of forearm vasculature (284).

SECONDARY HYPERTENSION AND ITS CAUSES

Most instances of hypertension (85% to 90%) have no known underlying cause and are thus called *primary* or *essential hypertension*. However, many different conditions may be associated with secondary hypertension. Examples of these conditions are listed in Table 20.2. The more common causes include renal parenchymal disease, renal artery stenosis, obstructive sleep apnea (OSA), and primary aldosteronism (PA) (3). Certain circumstances should prompt consideration of secondary forms of hypertension including rapid onset, severity of hypertension, resistance to antihypertensive drug therapy, absence of family history, signs of vascular disease elsewhere, onset after age 60, or before 30 years (285). Recommended routine laboratory evaluation for secondary forms of hypertension includes hematocrit, serum potassium, serum creatinine, blood urea nitrogen, urinalysis, UAE, fasting blood glucose, serum cholesterol, plasma triglycerides, plasma renin activity, urinary sodium and potassium, electrocardiography, and possibly echocardiography (77). Additional studies such as polysomnography for OSA or Doppler studies or angiography for renal artery stenosis may be required (3).

TABLE 20.2 Secondary forms of hypertension

- Renal parenchymal disease
- Renal artery stenosis
- Endocrine abnormalities
  - Adrenal gland
    - Pheochromocytoma
    - Cushing syndrome
    - Primary aldosteronism
    - Other mineralocorticoid disorders
    - Congenital hyperplasia
  - Hyperparathyroidism
  - Hyperthyroidism
  - Paragangliomas
- Pregnancy related
  - Preeclampsia
  - Eclampsia
  - Postpartum acute renal failure
- Neurologic disorders
  - Intracranial tumor
  - Cerebrovascular disease
  - Encephalitis
  - Acute porphyria
  - Neurofibromatosis
- Miscellaneous
  - Obstructive sleep apnea
  - Licorice ingestion
  - Various medications
    - Oral contraceptives
    - Calcineurin inhibitors
    - Antidepressants
    - Steroids
    - Cocaine
  - Coarctation of the aorta
  - Carcinoid syndrome

Prevalence of Secondary Hypertension

Secondary hypertension represents only a small percentage of cases of hypertension, reported in various studies from 5% to 21% (Table 20.3) (286–292). The differences undoubtedly are due to variation in referral patterns to hypertension study centers where the studies are performed. The frequency of secondary hypertension also depends on the demographics of the population studied. Anderson et al. (286) showed that the prevalence of secondary hypertension increased with advancing age and prevalence of atherosclerosis. The leading causes were renal artery stenosis and renal parenchymal disease. Increased use of the plasma aldosterone concentration/plasma renin activity has led to an increased detection of PA as a secondary cause of hypertension accounting for as much as 10% of all hypertension (293). The prevalence of OSA is also increasing as a cause of secondary hypertension due to the current obesity epidemic (294). The most frequent causes of secondary hypertension in children are renal parenchymal disease and administration of drugs (295).

The prevalence of secondary causes of hypertension is also greater in patients with malignant hypertension. A study performed by Sinclair et al. (292) in Australia examined 83 patients with malignant hypertension. These authors (292)

TABLE 20.3 Frequency of secondary forms in patients with hypertension

Authors	Number of patients	Secondary hypertension (%)	Renal parenchymal disease (%)	Renovascular disease (%)	Other (%)	Oral contraceptives (%)
Danielson and Dammstrom (289)	1000	4.7	2.1	1	0.8	0.8
Berglund et al. (288)	689	5.8	3.6	0.6	1.6	—
Sinclair et al. (292)	3783	7.9	5.6	0.7	0.6	1
Gifford (290)	4939	11.1	5.2	5.1	0.8	—
Bech and Hilden (287)	482	20.7	12.6	5	1.6	1.5
Anderson et al. (286)	4429	10.7	1.8	3.1	5.8	—
Omura et al. (291)	1020	9.1	0[a]	0.5	8.6	—

[a]Excluded patients with renal failure.

found that 80% of their group had secondary hypertension, with 65% related to renal parenchymal disease and 13% due to renovascular disease. Lip et al. (87) studied 242 patients with malignant hypertension over a 23-year period. These investigators found that secondary causes of hypertension were present in 40% of their patients. Secondary causes included chronic renal failure of unknown origin, glomerulonephritis, polycystic kidney disease, renal artery stenosis, PA, pyelonephritis, and pheochromocytoma. These studies indicate that some patients with malignant hypertension, at least those with renal artery stenosis, may have a potentially curable lesion.

Renal Artery Stenosis—Atherosclerotic Renovascular Disease

Many conditions are recognized as causes of renal artery stenosis (Table 20.4). By far, the most common are atherosclerosis, which occurs in 90% of cases, and fibromuscular dysplasia (FMD), which is seen in most of the remaining cases (296–298). Atherosclerotic renovascular disease and associated findings such as cholesterol emboli are sufficiently common to warrant detailed discussion. I will then comment on the other causes of renal artery stenosis including FMD, renal artery aneurysms, and Takayasu disease.

TABLE 20.4 Causes of renal artery stenosis

Atherosclerosis
Renal artery dysplasia
Dissecting aneurysms of either the aorta or the renal artery
Other aneurysms
Takayasu arteritis
Other arteritides
Neurofibromatosis
Thromboemboli
Moyamoya disease
Direct compression by tumor
Irradiation
Trauma
Arteriovenous fistulas

Clinical Features

Atherosclerotic renovascular disease (ARAS) is a disease of aging and is more common in those with evidence of atherosclerosis affecting extrarenal vessels as well (296,297,299,300). In autopsy studies, the prevalence of significant renal artery stenosis ranges between 5% and 42% depending on age (296,300). A population-based study of 800 people older than 65 years without known kidney disease showed a prevalence of ARAS of 6.8% (greater than 60% occlusion based on Doppler study) (301). The prevalence of ARAS in patients undergoing angiographic studies of other vessels such as coronary angiography ranges between 14% and 30% (297,300). Patients with extensive peripheral vascular disease have prevalence rates of renal artery involvement as high as 50% (302). Bilateral renal artery stenosis may be present in 33% to 39% of cases with ARAS (292). Clearly, patients with bilateral involvement have a higher incidence of renal failure. Renal artery stenosis is more often seen in men than in women (299). It should be noted that the true prevalence rate of ARAS is not known. Most of the studies have been performed in groups with known risk factors, and the definition of the degree of stenosis required for diagnosis as well as the methodology for measuring stenosis vary from one study to another.

Certain features of the clinical presentation in patients with hypertension suggest the possible presence of renal artery stenosis. The American College of Cardiology and the American Heart Association produced guidelines concerning various aspects of peripheral artery disease (including the renal arteries). Among these principles, they suggested clinical clues for the presence of ARAS. These included onset of hypertension before age 30 years or severe hypertension after age 55 years, malignant, resistant or accelerated hypertension, development of new azotemia or worsening renal function after administration of ACE inhibitor or angiotensin receptor blocker, unexplained atrophic kidney or size differential greater than 1.5 cm between kidneys, sudden unexplained pulmonary edema, unexplained renal dysfunction, multivessel coronary artery disease or peripheral arterial disease, and unexplained congestive heart failure or refractory angina (303). If renal parenchymal injury has occurred, then proteinuria may also be present (296). The proteinuria is usually mild, but nephrotic-range proteinuria may be seen (296).

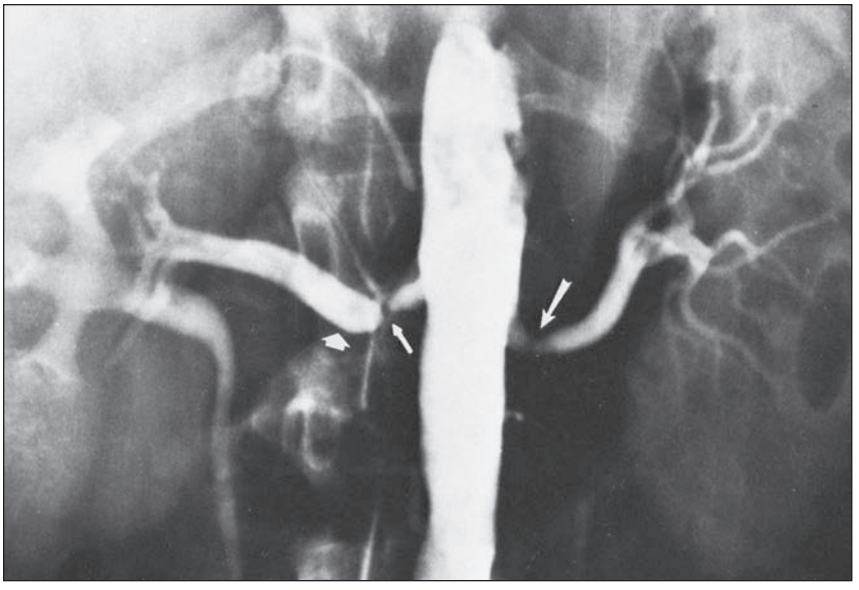

FIGURE 20.29 Abdominal aortogram showing severe stenosis (*short white arrow*) with poststenotic dilation (*white arrowhead*) of the early part of the right main renal artery. Stenosis is less severe (*long white arrow*) in the proximal part of the left main renal artery. (Courtesy of Dr. Olga Gatewood, Department of Radiology, Johns Hopkins Hospital, Baltimore, MD.)

A common complication of atherosclerosis is the occurrence of atheroemboli. This syndrome may occur spontaneously in as many as 23.5% of cases (304); however, it is most frequently seen following angioplasty or vascular surgery (305,306). Other risk factors include male gender, thrombolytic therapy, oral anticoagulants, and abdominal aortic aneurysms (305,306). The risks from anticoagulation and fibrinolytic therapy have been disputed (307). A clinical systemic syndrome has been associated with the showering of multiple cholesterol emboli into the renal vessels and other major branches of the aorta (305–307). Clinical signs include livedo reticularis, acute renal failure, hypertension, leg pain, gastrointestinal symptoms, and vision loss (305–307). Peripheral eosinophilia and decreased serum complement have also been associated with this syndrome. Severe systemic involvement usually presents within 7 days of a triggering event (305). A subacute subset with renal predominant involvement may occur weeks to months following the event with progressive loss of renal function. A chronic subset has also been described with slowly progressive loss of renal function following multiple embolic showers (305). The prevalence of these various manifestations is difficult to establish because many cases of atheroembolic disease may not be detected clinically and renal biopsy may be required (305,307). Spontaneous atheroembolism may present as unexplained renal failure or as de novo onset of hypertension (307).

Diagnostic Tests

The number and variety of tests used to assess the presence and extent of renal vascular lesions are beyond the scope of this chapter and are reviewed elsewhere (308–310). The gold standard among imaging techniques is catheter angiography with pressure gradient measurements; however, it is invasive and is used chiefly for assessment for renal revascularization (310). Noninvasive tests used for screening include magnetic resonance angiography, color-enhanced computed tomographic angiography, and ultrasonography (308–310). The noninvasive tests allow for an evaluation of function; however, the accuracy of those evaluations has not been proven (309).

Pathologic Findings

Renal Artery

The atherosclerotic plaque is usually present in the portion of the renal artery nearest the aorta, or it may be in the aorta and override the ostium of the renal artery. This lesion is recognized by abdominal aortography (Fig. 20.29). If the vessel is severely narrowed, poststenotic dilation is also present.

The changes are the same as those seen in systemic atherosclerosis and are characterized by the presence of a fibrous plaque, usually in the proximal third of the renal artery. Cross-section shows the fibrous plaque as an eccentric thickening of the intima (Fig. 20.30). The thickened intima contains amorphous material with lipid-laden macrophages, myofibroblasts, and various matrix

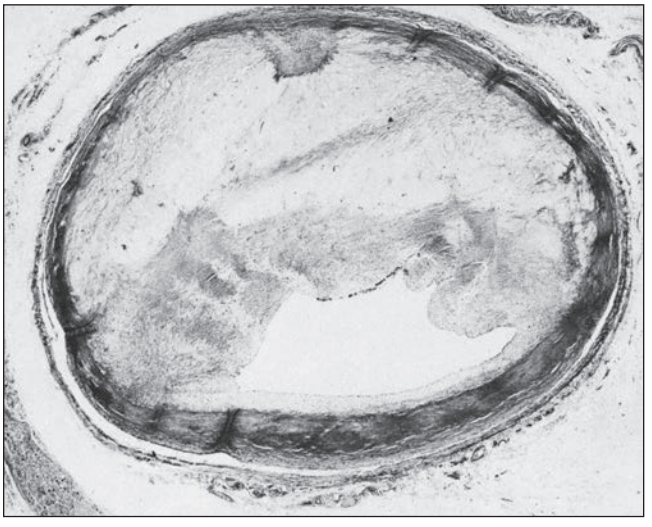

FIGURE 20.30 Section through the origin of a renal artery to show occlusion of the lumen by atherosclerosis, from a 50-year-old man with severe hypertension. (H&E, ×13.) (From Heptinstall RH. In: Mostofi FK, Smith DE, eds. Vascular Diseases of the Kidney. International Academy of Pathology Monograph Series. Baltimore: Williams & Wilkins, 1966.)

proteins. The endothelium is usually intact, but if it becomes disrupted, then platelet aggregation and thrombosis may ensue. The media underlying the plaque is frequently thinned relative to the uninvolved portion of the vessel. Major complications include saccular or dissecting aneurysm formation and the occurrence of cholesterol emboli. Both events are discussed later, but suffice it to say, their incidence is increased when a catheter has been placed into the renal artery. The atherosclerotic lesion is most frequently single; however, on occasion, multiple plaques are present, or a greater length of artery may be involved. Ostial plaques have a similar histologic appearance, but they are not as amenable to percutaneous angioplasty as intrarenal artery lesions.

Ipsilateral Kidney

The changes seen in kidneys distal to renal artery stenosis vary with the age of the patient. In addition, the location of the narrowing is important. If the main renal artery is involved, then the whole kidney will show ischemic changes. On the other hand, if a segmental branch of the renal artery is affected, the portion of the kidney supplied by that vessel will be ischemic, and the rest of the kidney will show changes consistent with exposure to higher systemic pressures, as described earlier.

In young patients, purer ischemic changes are present. Thus, the kidney is uniformly reduced in size, with a smooth surface with tubular atrophy as the predominant alteration. Two types of atrophic tubules are seen in the patient with hypertension. The "classic" atrophic tubules with thickened basement membranes derive from the proximal tubules and are thought to result from repeated tubular injury, with regeneration causing the multiple layers of the tubular basement membrane (311). A second type of atrophic tubule is the so-called "endocrine" form, first described by Selye and Stone (312) in hypertensive rats. In this form, the tubules have clear epithelial cells that are filled with mitochondria and narrow lumina, and they occur in clusters. They are usually derived from distal tubules (311). They are seen in association with ischemia. The glomeruli appear normal, but they are closer together than usual because of the tubular atrophy and loss. These glomeruli may be atubular, as discussed in the next paragraph. Although the interstitium may appear normal, a connective tissue stain demonstrates diffuse but fine fibrosis.

In elderly patients, these changes are usually superimposed on those of both aging and atherosclerosis. Thus, such kidneys are often small, have a granular surface, and, sometimes, larger scars, which may represent remote infarcts. Light microscopic examination reveals predominantly classic tubular atrophy and loss, with more interstitial fibrosis than seen in younger patients. Glomeruli may be normal, or they may show wrinkling and either partial or complete collapse of capillary loops. Simplification of the glomerular tuft is present with an apparent increase in the Bowman space. Vessels show a spectrum of changes, from hyaline arteriolosclerosis to fibrous intimal thickening in larger arteries. Hypertensive changes and cholesterol emboli may also be seen (313). These changes were confirmed in a study by Marcussen (314) in which he examined kidneys removed for renal artery stenosis. The ipsilateral kidneys from patients with renal artery stenosis were reduced in size; most were less than half the normal weight. Juxtaglomerular hyperplasia was only mild to moderate. Tubular atrophy was prominent, accompanied by interstitial fibrosis and crowding of the glomeruli. Sometimes, the Bowman space was so dilated as to form microcysts. Marcussen (314) documented the presence of atubular glomeruli, which are defined as glomeruli with open capillary loops that, on serial sectioning, are not attached to a tubule. When serial sections cannot be performed, the presence of atubular glomeruli may be suggested by the finding of surrounding atrophic tubules with or without periglomerular fibrosis (Fig. 20.31). In the controls, 95.9% of glomeruli were connected to proximal tubules, 1.5% were connected to atrophic tubules, and 2.5% were atubular. In contrast, in the patients with renal artery stenosis, 8.1% of glomeruli were connected to proximal tubules, 39.9% were connected to atrophic tubules, and 52% were atubular. Thus, in these patients, most glomeruli were either atubular or connected to atrophic tubules. This investigator also found that proximal tubules were more frequently atrophic.

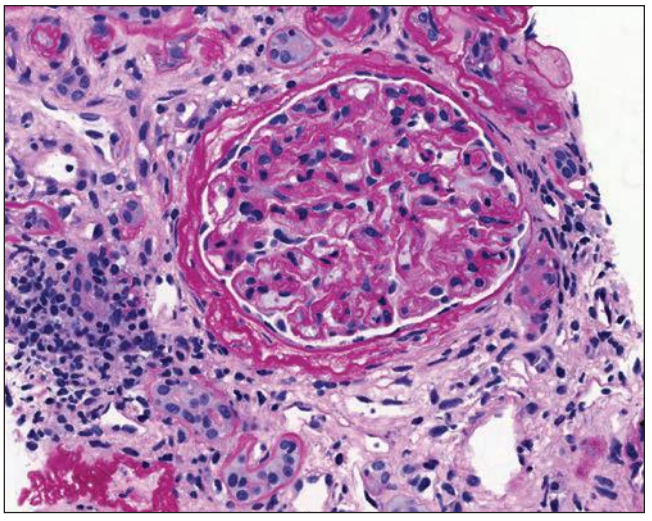

FIGURE 20.31 Atubular glomerulus showing open capillary loops and periglomerular fibrosis. Also note that the glomerulus is surrounded by atrophic tubules and interstitial fibrosis. (PAS, ×300.)

Cholesterol Emboli

Cholesterol emboli have been reported in renal vessels at autopsy (306) with an incidence ranging from 0.1% to 3.3%. However, if one looks only at the population at risk, the frequency is much higher; such emboli are present at autopsy in 31% of patients with aortic aneurysms and in 77.3% of patients dying during or shortly after surgical procedures involving the abdominal aorta (306). Involved vessels may range from arcuate arteries to glomerular capillary loops. Cholesterol emboli may also be found in the kidneys removed for renovascular hypertension (313). These emboli are characterized by needle-like spaces representing the cholesterol crystals embedded in amorphous debris. The clefts are quickly surrounded by giant cells and incite a fibrous reaction (Fig. 20.32). Polarized light of unstained sections may be used to accentuate the cholesterol emboli. The emboli are frequently present at bifurcations. The renal parenchyma beyond the affected vessels shows alterations resulting from ischemia with atrophy predominant over infarcts.

Etiology and Pathogenesis

Goldblatt et al. (315) showed that constriction of one artery in the dog led to persistent elevation in blood pressure, a model analogous to human unilateral renal artery stenosis. It was later found that the initial elevation was due to increased

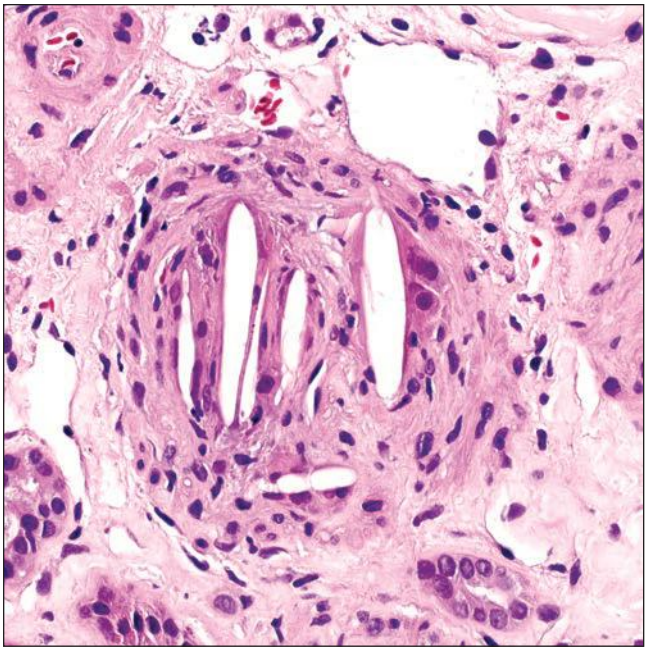

FIGURE 20.32 Cholesterol clefts in the lumen of an interlobular artery. Cholesterol is dissolved during the processing of the section, leaving characteristic clefts. (H&E, ×365.)

plasma renin activity and peripheral resistance. In humans, it has been found that a pressure gradient of 20 to 25 mm Hg corresponding to a critical stenosis of 70% to 80% is required to reduce blood flow to a sufficient level to raise renin in the renal vein (309,316). Increased AII leads to increase in aldosterone, which results in sodium retention, hypervolemia, and increased cardiac output, resulting in hypertension. The injury to the kidney is due in part to the ischemia from the decreased perfusion. However, the degree of renal artery stenosis does not predict the degree of renal injury nor the response to angioplastic stenting suggesting that other factors may be important (317). In humans with atherosclerotic renal artery stenosis, the kidney is not without blemish. Rather, the atherosclerosis has developed gradually on an aging kidney that is often further compromised by smoking, obesity, or diabetes. The vessels are not able to autoregulate, and when the "critical stenosis" occurs, the structural changes in the vessels such as remodeling have already begun enabling maintenance of the hypertension. The reduced perfusion leads to apoptosis, shrinkage, and atrophy of the tubules. Furthermore, activation of the RAS activates oxidative stress mechanisms that lead to interstitial fibrosis. Additional risk factors such as elevated cholesterol and endothelial dysfunction act synergistically to increase tubulointerstitial injury and microvascular rarefaction (296,317,318). Textor suggests that these repeated small injuries set the substrate for progressive renal injury (309).

The extent of this underlying renal parenchymal injury may predict the response to revascularization. The study by Grone et al. (319) examined the potential reversibility of tubular atrophy using an experimental model of ischemic injury. These investigators defined the term *hibernation* as a state of reduced organ function at rest that can be reversed by improving blood flow or increasing workload. As has been observed, renal artery stenosis leads to a reduction in the size of the kidney and atrophy, partly because of reduction of the blood flow. In some cases, revascularization of the kidney may result in return of at least some function to the kidney. In this study, the authors induced renal ischemia in rats using a clip on the renal artery. This procedure resulted in atrophy, which reversed with reestablishment of blood flow. Some of the animals received ACE inhibitors to induce renal tubular atrophy. All of the groups receiving ACE inhibitors had tubular atrophy predominantly in the proximal tubules with reduction in renal function. The tubules showed a loss of morphologic differentiation and decrease in the activity of various lysosomal and brush border enzymes. Atrophy was reversible after 14 days of ischemia even in those groups with more severe atrophy induced by the addition of ACE inhibitors. These researchers suggested that the presence of atubular glomeruli may be a useful prognosticator of potential irreversibility of renal injury in this setting (319).

Differential Diagnosis

Fibromuscular Dysplasia

FMD is the second most common cause of renal artery stenosis. This disease affects a younger population usually women, most commonly presenting in the second and third decades (298,320,321) compared with atherosclerosis, which presents in patients beyond the fourth decade. FMD has been described in the elderly as well (322). This vascular lesion most commonly affects the renal (79.7%), extracranial carotid (74.3%), or vertebral (36.6%) arteries; however, any vascular bed may be involved (320,321). Of the patients who had imaging of two or more vascular beds, 35.3% had FMD in two beds, 21.9% in three beds, and 9.1% in four beds (320). Studies involving screening of potential renal donors have documented that the prevalence of this lesion in the renal artery ranges between 3.8% and 6.6% in the general population (298,323). One study suggests that intrarenal vessels may also be involved (324). These investigators presented a case of a 58-year-old male patient with medial fibroplasia with aneurysms who required nephrectomy. Examination of the intrarenal vessels demonstrated continuation of the variation in medial thickness to the arcuate arteries and into some interlobular arteries. These authors suggested that such involvement could account for hypertension after correction of renal artery lesions.

Pathologic Findings The classification of the lesions of renal artery dysplasia as described by Harrison and McCormack (325,326) has not been further refined. The classification based on the layer of the vessel wall affected is as follows:

I. Intimal fibroplasia
II. Medial types
    A. Medial hyperplasia
    B. Medial fibroplasia with aneurysms
    C. Perimedial fibroplasia
    D. Medial dissection
III. Adventitial fibroplasia

Determination of the type of renal artery dysplasia requires both longitudinal and cross sections of the involved vessels, so all the layers of the vessel wall can be evaluated. Trichrome and elastic stains such as Verhoeff-van Gieson are particularly useful in the assessment of these lesions.

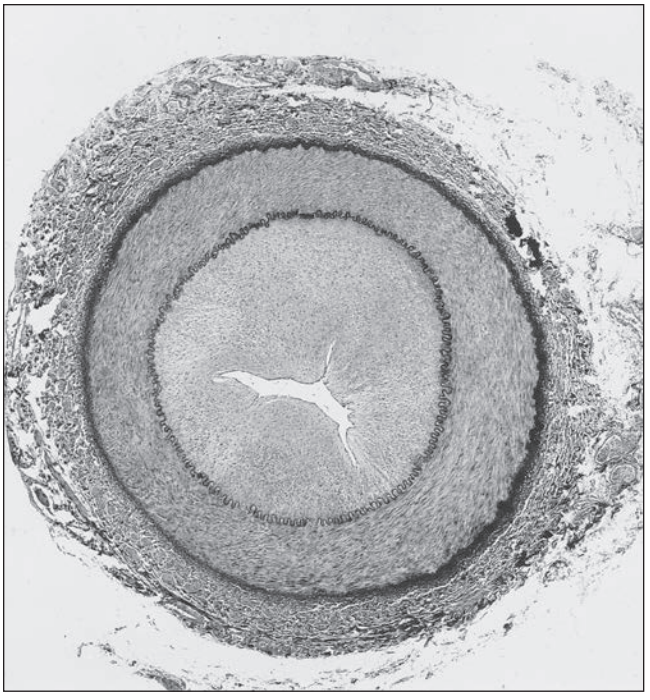

FIGURE 20.33 Transverse section of the renal artery with primary intimal fibroplasia. The lumen is severely reduced by accumulation of loose cellular material. (H&E, ×25.) (Courtesy of Dr. L. McCormack.)

Intimal fibroplasia is rare, accounting for only 1% to 2% of all cases of renal artery dysplasia (327). The involved vessels show a circumferential accumulation of loose, moderately cellular fibrous tissue inside the internal elastic lamina (Fig. 20.33). Neither lipid nor inflammatory cells are noted. Radiographic studies show a smooth, segmental, stenotic lesion. This lesion may affect other major branches of the aorta and is often bilateral. Intimal fibroplasia may also be seen in the other forms of renal artery dysplasia as a secondary change.

Medial hyperplasia is characterized by true hyperplasia of the smooth muscle cells of the media (Fig. 20.34). This condition results in narrowing of the lumen, which radiographically is manifest as a smooth, linear stenosis without aneurysm. This type accounts for 5% to 15% of cases and is detected most commonly in the second decade in women and in men between the ages of 35 and 45 years (327).

Medial fibroplasia with aneurysms is the most common form, accounting for 60% to 70% of cases (327). It occurs in the distal two thirds of the renal artery and its major branches and in women aged 25 to 50 years and is bilateral in 60% of cases. Thus, it is also the most common form to show bilateral disease. The lesion is characterized by thickened ridges caused by replacement of smooth muscle by collagen. Aneurysms form as a result of loss of smooth muscle and a deficient elastic lamina. In involved segments, the stenoses correlate with the ridges and alternate with areas of thinning, which represent the aneurysms (Fig. 20.35). Thrombosis or rupture occurs only rarely. The typical radiographic picture resembles a string of beads or sausages (Fig. 20.36).

Perimedial fibroplasia is the second most common type of dysplasia, accounting for 15% to 25% of renal artery

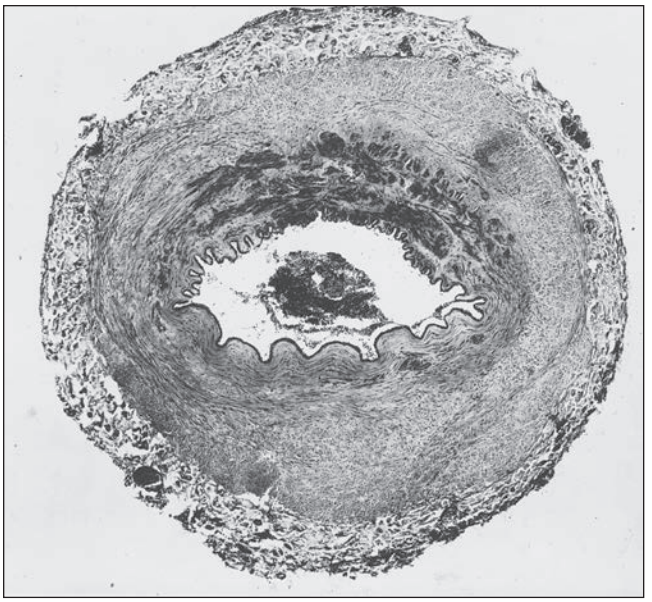

FIGURE 20.34 Transverse section of the renal artery to show medial hyperplasia characterized by a random disorganized increase in muscle cells in the media with a reduction of the lumen. (H&E, ×25.) (Courtesy of Dr. T. A. Stamey.)

dysplasia. It occurs most commonly in women between the ages of 15 and 30 years. The lesion is characterized by replacement of the outer two thirds of the media by dense collagen (Fig. 20.37). The multifocal stenoses produce irregular beading such that, on radiographic examination,

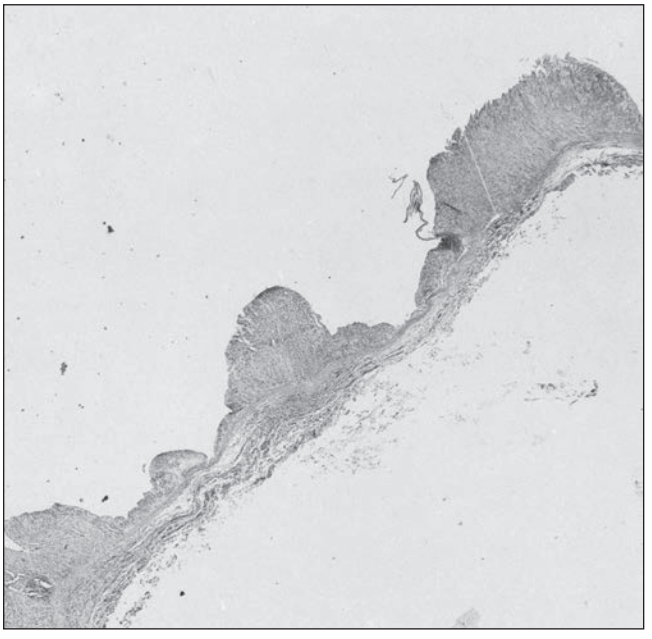

FIGURE 20.35 Medial fibroplasia with multiple aneurysms with alternating segments of increased musculofibrous tissue and marked attenuation of the muscularis. Longitudinal section. (H&E, ×6.) (Courtesy of Dr. J. C. Hunt.)

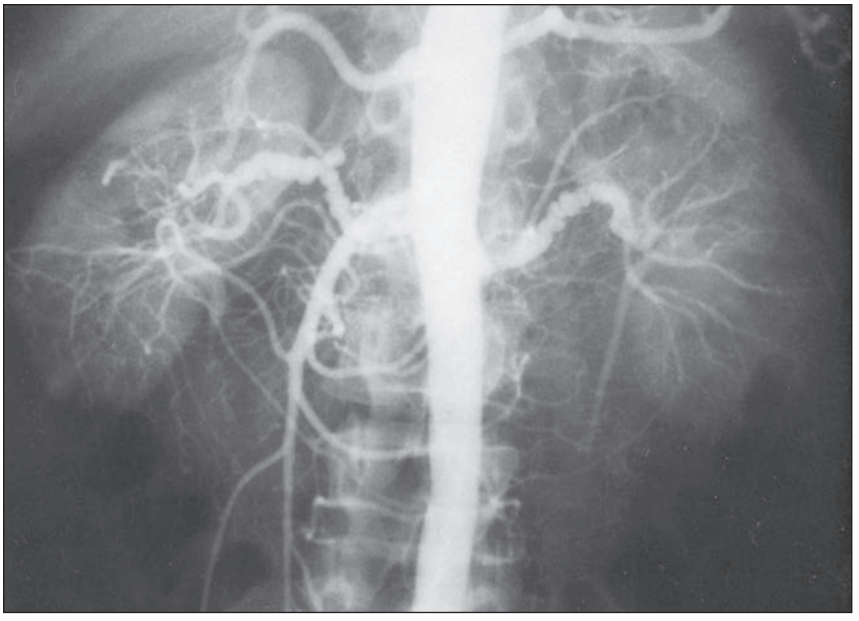

FIGURE 20.36 Aortogram to show a sausage string, or string of beads, appearance of both main renal arteries in a case of medial fibroplasia with multiple aneurysms. Such a case would exhibit the pathologic features shown in Figure 21.33. (Courtesy of the Department of Radiology, Johns Hopkins Hospital, Baltimore, MD.)

the beads are smaller than the vessel diameter (327). Rapid increase in hypertension is frequently seen. Thrombosis is more common in this form than in the other types of renal artery dysplasia. The Masson trichrome stain is particularly helpful in distinguishing this form of dysplasia from the other forms.

Medial dissection accounts for 5% to 10% of cases. The channel forms in the outer one third of the vessel wall. The initial defect is thought to lie in the internal elastic lamina and provides an access for blood to enter the media (326). Intimal fibroplasia may occur in this lesion in the area of the dissection. Renal infarcts are more common in this lesion than in the other types of renal artery dysplasia.

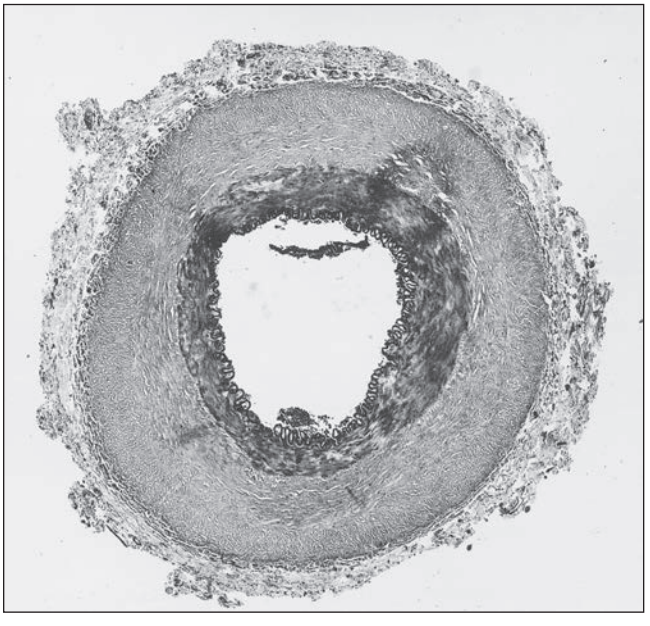

FIGURE 20.37 Perimedial fibroplasia type of renal artery dysplasia in which the outer half to two thirds of the media is replaced by fibrous tissue. (H&E, ×32.)

Periarterial fibroplasia is rare and accounts for less than 1% of all types. It is manifest by dense collagen surrounding the vessel extending from the adventitia that penetrates the fibrofatty tissue. Similar lesions may be seen outside the renal arteries elsewhere in vessels of the same size. It has also been compared to idiopathic retroperitoneal fibrosis (325).

Pathogenesis The pathogenesis of these lesions continues to be unknown. The FMD Registry was established in 2008 with the hope that greater understanding of these lesions could be attained. Olin et al. (320) reported on the clinical features, presenting symptoms, and vascular events in the first 447 patients accrued. Genetic factors may be important as there is an increased risk of the disease in first-degree relatives (320,321). In the Registry data, 7.3% of patients had a family member with confirmed diagnosis of FMD. A high prevalence of stroke (53.5%), aneurysm (23.5%), and sudden death (19.6%), all commonly associated with FMD, was also noted among family members (320).

Clinical Course and Therapy Catheter-based angiography is the best method for diagnosis and allows for determination of the severity of the pressure gradient (298). Several studies using repeated angiograms have been performed on patients with various forms of renal artery dysplasia. One such study showed that many patients progress with narrowing of their renal arteries when followed up to 10 years by angiographic techniques (328). Percutaneous transluminal renal angioplasty (PTRA) is the current treatment of choice for this condition (298,329). Alhadad et al. (329) completed a retrospective study of 69 patients with renal artery dysplasia, 59 of whom had PTRA. The procedure was technically successful in 95% of the patients. Follow-up with a mean of 7 years showed cure in 24% with long-term benefit in up to 63% of the patients. Patients with longer periods of hypertension prior to the procedure had the poorer outcomes. Carmo et al. (330) used surgical revascularization for those patients who failed PTRA and

with a 1-year follow-up show similar results with 24% cure of hypertension and 64% with benefit. Stents may be used in those patients with aneurysms (298).

Renal Artery Aneurysms

Bulbul and Farrow (331), in a review of renal artery aneurysms, found that the incidence varied between 0.01% and 0.1%. Browne et al. (332) found a prevalence of 1% for renal artery aneurysms during a retrospective review of contrast-enhanced magnetic resonance angiography in screening for suspected renovascular disease. Bulbul and Farrow studied 67 patients with renal artery aneurysms and found a slight male predominance (3:2), with a range in age of 20 to 76 years. The presentation included hypertension (55%), hematuria (30%), flank pain (21%), and, in one patient each, gastrointestinal bleeding, polyarteritis nodosa, and renal failure. Ninety-two percent of these aneurysms occurred in the extrarenal portion of the renal artery, with 70% saccular, 22.5% fusiform, and 7.5% dissecting aneurysms. All but seven were less than 2 cm in diameter. Most were not calcified. Twenty percent were associated with medial hyperplasia. Rupture of these aneurysms is rare, particularly if they measure less than 1 cm except that there is an increased risk of rupture during pregnancy (331,332). Indications for repair include refractory hypertension, severe hematuria, flank pain, dissection, size greater than 2 cm, or progression in dilation (333). Surgical repair is suggested for those who are good surgical candidates as the long-term prognosis is very good (334). In the past decade, endovascular techniques have been developed that are also promising. These are reviewed in Elaassar et al. (333) including the criteria that determine which approach is most advantageous.

Dissections of the aorta may extend into the renal artery and may result in either hypertension or renal failure. Dissections of the renal artery may also occur after trauma, renal artery catheterization, or spontaneously. In the study conducted by Bulbul and Farrow (331), three of the five dissecting aneurysms were associated with catheterization. The presenting symptoms included the sudden onset of flank pain and hypertension.

Takayasu Disease

Takayasu disease affects the aorta and its major branches by an ill-defined inflammatory process elicited by an unknown trigger. A pathogenetic mechanism has been proposed suggesting that cell-mediated immunity is the principal effector (335). Criteria for its diagnosis include onset at or under 40 years of age, claudication of an extremity, decrease in brachial pulse, greater than 10 mm Hg difference in systolic blood pressure between arms, bruit over the subclavian artery, and narrowing or occlusion of the aorta, its primary branches, or major arteries of the extremities (336). The presence of three of these six criteria is highly specific and sensitive for the disease. The disease may be restricted to the arch of the aorta, it may involve the entire aorta, or it may be present in the abdominal aorta or its branches (337,338). Hypertension, when present, is due to either coarctation of the aorta or renal artery stenosis (Fig. 20.38). This disease was originally described in Japan but has now been described in most ethnic groups (338–340). Takayasu disease affects predominantly young women at a mean of 30 years (range, 5 to 54 years) (339,340). The clinical features included malaise, claudication, fever, dizziness, headache, bruits, absent pulses, and hypertension. Angiographic abnormalities included occlusion, stenosis, irregularity of the lumen, or ectasia or aneurysm, often involving multiple sites. Renal artery involvement was noted in 15% to 25% of patients (339,340). A study in South Africa (338) reviewed 272 patients with a mean age at presentation of 25 years and a female predominance. Most were of mixed race or Asian background (62%) with 30% black and 8% Caucasian. Hypertension was the most common presenting sign, and 40% had renal artery stenosis. PTRA was used in 10 patients with good results in only 3.

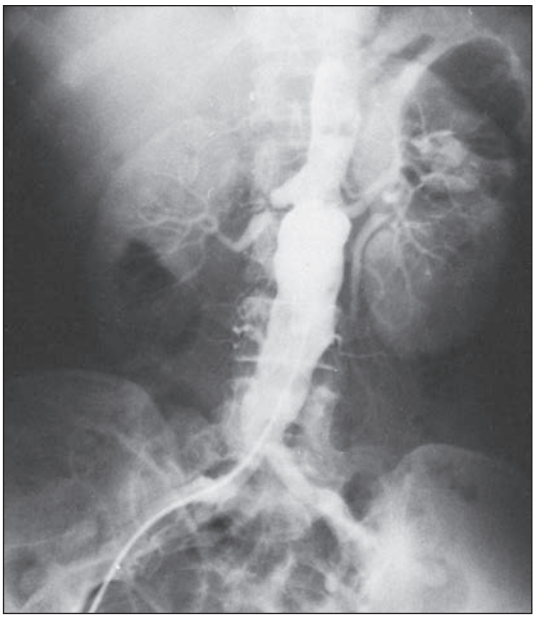

FIGURE 20.38 Abdominal aortogram from a 45-year-old woman with Takayasu syndrome showing aneurysmal dilation of the abdominal aorta. Marked narrowing in the proximal part of the right renal artery is demonstrated. The right kidney is smaller than the left, indicating the hemodynamic severity of the arterial stenosis. (Courtesy of Dr. T. Ideura.)

Takayasu disease is characterized by an acute phase with granulomatous arteritis that affects the media and adventitia with an inflammatory infiltrate composed of lymphocytes, plasma cells, histiocytes, and occasional giant cells (337). More recent studies have shown that the inflammatory infiltrate is complex involving $CD8^+$ T cells, NK cells, γδ T cells, dendritic cells, macrophages, $CD4^+$ T cells, and Th17 cells (335). Neovascularization is a prominent feature from the intima to the vasa vasorum (337). Sclerosis then ensues with intimal hyperplasia, medial degeneration, and adventitial fibrosis. Disruption of the elastic lamellae may be seen, and thrombosis and aneurysm formation have been noted as in other forms of vasculitis. The result of these structural alterations may be so severe as to cause virtual coarctation of the aorta. The histologic changes are similar to those of giant cell aortitis, but the prevalence of abdominal aortic and renal artery involvement is typical of Takayasu disease (337).

Corticosteroids are the mainstay of treatment with angioplastic and surgical therapy as needed for severe vascular lesions (340). Outcome depends on early recognition of the possibility of the disease from clinical symptoms as the radiologic techniques have become quite advanced and provide excellent support in its confirmation (341).

Miscellaneous Causes

The causes of renal artery stenosis are listed in Table 20.4. *Neurofibromatosis* (von Recklinghausen disease) is occasionally associated with hypertension with a prevalence of 7% to 58% usually because of renal artery stenosis, although coarctation of the aorta may also occur (342). The mean age at which hypertension appears in such cases is 11 years (343). Smaller vessels show mesodermal dysplasia, which is a nodular proliferation of cells demonstrated by immunohistochemistry and electron microscopy to be smooth muscle cells (344). These nodules may be present in the media or in the intima and may result either in stenosis or in occlusion (Fig. 20.39). These proliferative nodules are common in neurofibromatosis, appearing in 7 of 18 autopsies of patients with this disease increasing in frequency with age (345). Hamilton and Friedman (346) suggested that these lesions arise due to excessive cellular proliferation in response to injury as the lesions are in a similar distribution to those seen in atherosclerosis. Hypertension in neurofibromatosis may also be due to these proliferative lesions in the aorta, lesions that cause coarctation of that vessel. In addition, patients with neurofibromatosis have an increased incidence of pheochromocytomas, which may also cause hypertension (343).

Moyamoya disease is a condition first described in Japan that affects the carotid arteries and its branches (347). It is characterized by occlusions or stenoses of these vessels with the formation of netlike vessels. The carotid arteries show fibrointimal thickening and medial thinning. Renal artery stenosis has been reported in 4% to 8% of patients with moyamoya disease (348). A study in children has reported a prevalence of renovascular hypertension in 8.3% of children with moyamoya disease (349). Intimal fibroplasia was seen in a specimen taken from one of the children. It should be noted that the network of vessels surrounding carotid arteries has not been described in radiologic studies of the renal arteries. Thus, it is not clear whether these lesions are due to the same defect present in the cerebral vessels.

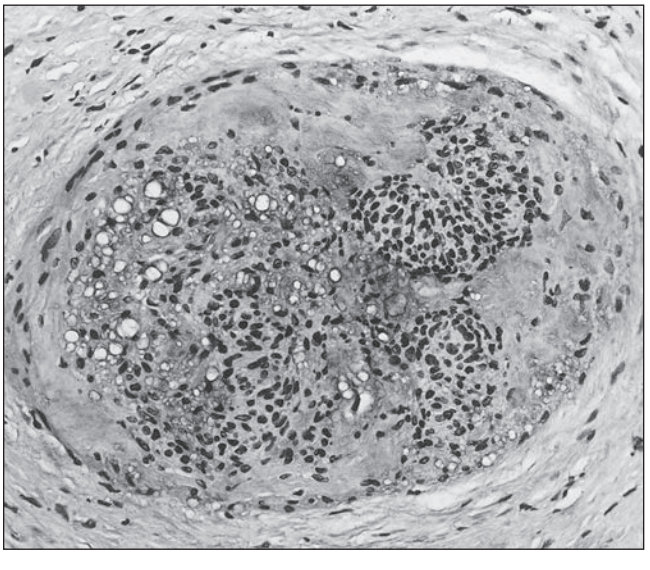

FIGURE 20.39 Intima of a small arcuate or large interlobular artery completely replaced by small cells with dense nuclei and by mucinous connective tissue. (H&E, ×203.) (Courtesy of Dr. Gary S. Hill.)

Segmental arterial mediolysis was first described in 1976 by Slavin and Gonzalez-Licea (350) at autopsy in three patients who had mesenteric ischemia and renal infarcts. It is now recognized as a usually self-limited vasculopathy involving small- to medium-sized mesenteric vessels and the renal circulation presenting with hemorrhage due to dissection or rupture of aneurysm (351,352). As the lesions heal, the vessels occasionally resemble the changes seen in FMD and may result in stenosis or occlusion of the vessel (351,352). The exact pathogenesis is uncertain, but vasospasm is considered to be an important factor (352). Hypertension caused by *direct compression of the renal artery by neoplasms* is discussed later. Effects of *radiation* on the kidney are discussed in Chapter 18. *Trauma* and *arteriovenous fistulas* have also been associated with hypertension.

Clinical Course, Prognosis, and Therapy

Clinical Course and Prognosis

This disease is usually silent and is often discovered during the investigation of other vascular conditions. The diagnosis of renovascular disease depends on the radiologic demonstration of stenotic lesions and on proof that these lesions are responsible for the loss of renal function. As stated earlier, the prevalence of significant renal artery stenosis has been shown to range between 15% and 22%. Studies prior to the introduction of statins showed significant rates of progression. For example, one typical study from the Cleveland Clinic (353) examined 85 patients who had had at least two angiograms over a period of 4 years. Forty-four percent of these patients showed progression of the lesion(s), with 16% going on to complete occlusion of the vessel. The latter occurred in the first 13 months in the patients with the most severe narrowing at the beginning of the study. It required ≤5 years for complete occlusion in patients with less severe narrowing. Kidney size was reduced more frequently in those patients who had progressive renal artery narrowing. The narrowing occurred in 62% of patients despite good control of blood pressure (353). The chief conclusion from most of the studies was that renal artery stenosis is a progressive disease, with occlusion occurring more commonly in those vessels with more severe degrees of intimal disease at the beginning of the study.

Rimmer and Gennari (354) reviewed five such studies. In the combined data, 237 patients were considered, of whom 49% showed worsening of degree of stenosis only, whereas 14% progressed to occlusion during a follow-up period of 6 to 180 months. Furthermore, these investigators found that control of hypertension had little effect on progression, and serum creatinine did not accurately predict progression. They calculated that the rate of progression fell in a range of 0.4% to 1.5% additional stenosis per month (354). Another study showed a higher rate of progression with overall cumulative incidence of progression 35% at 3 years and 51% at 5 years (355). Those patients with more severe disease at baseline were more likely to progress. Since these patients inevitably have atherosclerosis affecting other vascular beds, they have considerable risk for cardiovascular mortality as well as for ESRD (356). More recent studies in which patients have received statins in addition to antihypertensive medications show a slower rate of progression (357). In one such retrospective study, 79 patients had repeat angiography with a mean follow-up time between angiograms of 27.8 months (358). The authors found that statin therapy significantly reduced the risk of progression of

atherosclerotic renovascular disease from 30% cumulative risk over 3 years for patients not receiving statins to 6% for those who did receive statins. Furthermore, regression was seen in 12 patients, most of whom were receiving statins (358).

In a recent review of the literature, risk factors for progression of atherosclerotic renal disease included uncontrolled systolic hypertension (greater than 160 mm Hg), diabetes mellitus, high-grade (greater than 70%) ipsilateral and contralateral atherosclerotic renal vascular disease (ARVD), and significant baseline proteinuria (359). In addition, they found risk factors for atrophy including systolic blood pressure greater than 160 mm Hg, greater than 60% renal artery stenosis, and decreased renal cortical blood flow. Finally, they demonstrated that risk factors for decreased GFR included abnormal baseline creatinine and bilateral ARVD or unilateral ARVD in a solitary kidney (359).

Therapy

Treatment of hypertension associated with renal artery stenosis has evolved over the years. Endovascular angioplasty most often with stent placement now accounts for greater than 97% of invasive procedures for correction of the vascular lesions with surgical intervention used only in cases not amenable to an endovascular approach (360). From 1992 to 2004, approximately 13.4% of patients underwent revascularization within 6 months of diagnosis (360). The question of medical management versus revascularization is still undecided despite some recent studies presented below. The major issue is the high mortality in these patients due to comorbidities.

Numerous studies have been undertaken over the last 30 years comparing various treatment modalities and differing measures of outcome. Review of these studies is beyond the scope of this chapter. Rather, I will discuss several papers emphasizing the newer literature comparing endovascular procedures to medical management. It should be noted that revascularization has been shown to benefit patients with FMD (361). The remainder of this discussion will focus on patients with renovascular atherosclerotic disease.

Numerous investigators have examined the effects of renal angioplasty on renal artery stenosis. Rimmer and Gennari (354) compared the results of angioplasty with those of surgical revascularization in a review of seven reports of these procedures. With angioplasty, 43% of patients improved, 57% were the same or worse, and 5% died. Renal revascularization produced similar results in this study, with 55% of patients improving, 31% remaining stable, and 14% becoming worse; 6% died. This study showed that angioplasty was at least equivalent to the surgical procedure. The placement of stents during angioplasty was introduced in the 1990s to prevent repeated stenosis of the renal artery. The stents are particularly useful when the narrowing is at the ostium, a location with a higher failure rate using PTRA. Beutler et al. (362) showed that stent placement stabilized renal function. However, complications did arise in some patients with renal failure directly attributable to stent placement in two patients. Thus, the question arose concerning whether revascularization provided additional benefit as compared to medical management.

Several studies have now compared these various techniques to medical management. Losito et al. (356) examined the course of disease in 195 patients who had angiographically demonstrated renal artery stenosis. Angioplasty and/or stent placement was used in 136 patients. Medical treatment was also used as appropriate during the follow-up period. Fifty-four patients received only medical therapy. A variety of antihypertensive agents were used, including ACE inhibitors, β-blockers, and calcium channel blockers. The mean follow-up time was 54 months. Multivariate analysis showed no difference in mortality or renal survival between the two treatment groups. Angioplasty was associated with better control of blood pressure, a finding repeated in many studies. Patients who received ACE inhibitors, across treatment groups, showed longer survival compared to other antihypertensive agents. Baseline serum creatinine was the only predictor of progression to ESRD.

Three recent randomized trials comparing endovascular repair to medical management have been established. The results have been reported for two of these (STAR and ASTRAL). The third has recently completed enrollment (Cardiovascular Outcomes in Renal Atherosclerotic Lesion [CORAL]). The Stent Placement and Blood Pressure and Lipid-lowering for the Prevention of Progression of Renal Dysfunction Caused by Atherosclerotic Ostial Stenosis of the Renal Artery (STAR) compared a group with stent placement at the renal ostium coupled with medical management of blood pressure to a group receiving medical management alone with regard to progression of renal failure. They found that 16% of the stent group and 22% of the medication-alone group had reached the primary end point of reduction in creatinine by 20% at the end of 2 years, but this result did not attain statistical significance (363). The Angioplasty and Stenting for Renal Artery Lesions (ASTRAL) trial also compared a group receiving revascularization and medication to a group receiving medication alone (364). The primary outcome was the renal function as determined by the inverse of the serum creatinine over time with a median follow-up of 33.6 months. The authors did not find a worthwhile clinical benefit between the two groups. This trial has been criticized because the degree of stenosis was determined centrally, the intervention technique varied (not all patients received stents), and patients were enrolled only if the local physician was uncertain of the appropriate management possibly introducing selection bias (309,365). The third randomized trial, the CORAL, was designed to avoid the selection bias of ASTRAL. In addition, patients with CKD have been included, and the protocols have been standardized among centers (365). Results are not yet published.

In summary, despite numerous studies comparing various therapies, we do not yet know which is optimal. One aspect of the problem is that no one knows how to determine how individual patients will fare with angioplasty. Simon has offered the following recommendations (365). Intervention is not recommended in patients with stable renal function. Currently, intervention may be considered in patients with bilateral renal artery stenosis and with congestive heart failure without obvious cardiac cause or with rapidly declining renal function, or with refractory hypertension (365).

Chronic Renal Parenchymal Disease

Chronic renal parenchymal disease is the most common cause of secondary hypertension in most series reviewed in Table 20.3. Hypertension may complicate any form of glomerular disease, as shown in several reviews (3,251). Some of the more common causes of renal parenchymal hypertension have included postinfectious glomerulonephritis, FSGS, IgA nephropathy, vasculitis, diabetes, crescentic glomerulonephritis, systemic

lupus erythematosus, polycystic kidney disease, and chronic interstitial nephritis. FSGS and membranoproliferative glomerulonephritis are particularly prone to an association with hypertension (251). It should be cautioned that it is difficult to determine the exact prevalence of chronic renal parenchymal disease as a secondary cause of hypertension as hypertension is both a cause and a complication of CKD. Crews et al. (366) found that the prevalence of CKD increased in groups with increasing levels of hypertension from 13.4% of those with normal blood pressure to 17.3% in those with prehypertension to 22% in patients with undiagnosed hypertension to 27.5% in those with known hypertension.

One potential mechanism for hypertension due to chronic renal parenchymal disease is increased intravascular volume followed by inappropriate activity of the renin-angiotensin-aldosterone system leading to sodium retention, and increased sympathetic activity (3,7). Hypertension occurs and a vicious cycle of continued renal injury ensues. Patients with CKD and hypertension have increased cardiovascular and cerebrovascular disease–related hospitalization (367). This increased risk suggests additional mechanisms involving inflammation and the immune system that may link all these forms of injury. These are reviewed in detail by Swaminathan and Shah (368).

Tumors and Other Conditions Associated With Hypertension

Pheochromocytoma

Pheochromocytoma is a tumor of chromaffin tissues in the adrenal glands. According to the World Health Organization (WHO) classification, tumors of chromaffin tissue elsewhere are termed paragangliomas (369). This tumor affects the two sexes equally and has a peak incidence in the fifth decade. The prevalence of this tumor in patients with hypertension is 0.1% to 0.6% (370). These tumors may be part of one of several familial syndromes as described below.

Familial Syndromes and Other Mutations

Approximately one third of the pheochromocytomas are due to 1 of 10 mutations with 3 first identified as familial syndromes (371). The first of these syndromes to be described was by Sipple (372) and included the association of carcinoma of the thyroid and pheochromocytoma. Sipple syndrome is now recognized as belonging to multiple endocrine neoplasia (MEN), type 2, an autosomal dominant syndrome. There are two variants that include pheochromocytomas: Type 2A includes medullary thyroid carcinoma with pheochromocytomas and parathyroid hyperplasia as well as cutaneous lichen amyloidosis. Type 2B refers to the combination of medullary thyroid carcinoma, pheochromocytomas, mucosal neuromas, and a marfanoid habitus (370). Activating mutations in the *RET* protooncogene, a receptor tyrosine kinase, cause the MEN syndromes by changes in regulation of cell proliferation and apoptosis (371,373). Most of the mutations are point mutations and lead to bilateral pheochromocytomas that are usually benign but may recur (371,373). The pheochromocytomas usually present in the fourth decade.

The other two syndromic associations with pheochromocytoma are von Hippel-Landau disease and neurofibromatosis-1 (NF-1) (371,373). Von Hippel-Landau disease is autosomal dominant and consists of various combinations of renal cell carcinomas and cysts, CNS and retinal hemangioblastomas, pheochromocytomas, pancreatic tumors and cysts, endolymphatic sac tumors, and epididymal cysts (370). Pheochromocytomas are present in about 15% to 25% of patients with the syndrome. Malignancy is only present in about 5% of the patients. The *VHL* gene on chromosome 3 is a tumor suppressor gene with missense mutations leading to loss of heterozygosity in a wild-type allele producing most pheochromocytomas within the syndrome (371,373). Patients with NF-1 (von Recklinghausen disease) have inactivating mutations in *NF1*, a tumor suppressor gene, and its protein neurofibromin producing constitutive activation of the Ras pathway and uncontrolled cell proliferation (371,373). The incidence of pheochromocytomas in the disease is 5% although autopsy studies have found up to 13% incidence (371,373). Approximately 10% are malignant.

Mutations in a number of other genes have also been associated with pheochromocytomas. Mutations in each of the four subunits of the succinate dehydrogenase (SDH) complex of the mitochondrial respiratory chain have been identified in pheochromocytomas (371,373). Paragangliomas of the head, chest, and abdomen are the most common tumors with these mutations. A higher rate of malignancy is also seen particularly with *SDHB* mutations (371,373). Three other genes have been identified in families with pheochromocytomas including kinesin family member 1B, a proapoptotic factor; transmembrane protein 127, a tumor suppressor gene; and MYC-associated factor X, a transcription factor (373). The tumorigenic mechanisms for each of these genes are reviewed by Welander et al. (373).

Clinical Features and Diagnostic Tests

Patients with pheochromocytomas represent 0.1% to 0.6% of all those with hypertension (370). Variable clinical manifestations have been reported, but the characteristic triad on presentation of headache, tachycardia, and diaphoresis in a patient with hypertension has a high sensitivity (90.9%) and specificity (93.8%) (293). These symptoms may last for only a few minutes or for hours. Hypertension is present in greater than 90% of patients with pheochromocytomas and is frequently paroxysmal (293,370). Most pheochromocytomas produce a mixture of norepinephrine and epinephrine. However, MEN-2– and NF-1–associated tumors always secrete epinephrine, and VHL-related pheochromocytomas produce norepinephrine (293).

Screening for pheochromocytomas should be considered in patients with resistant hypertension, the classic triad described above, family history, hypertensive reaction to anesthesia, early onset of hypertension, incidentally found adrenal tumors, or those with the genetic syndromes listed above (374). Hypertension in pheochromocytomas results from release of catecholamines into the circulation. The tumor cells consistently metabolize the catecholamine to metanephrine and norepinephrine. Thus, the most sensitive techniques currently used are measurements of plasma-free metanephrines or urinary-fractionated metanephrines. The sensitivity is 99% and specificity is 89% for the plasma measurement and 97% and 86%, respectively, for the urinary determination (370,374). Renal function may affect urinary determinations. A clonidine suppression test can be used to eliminate false positives (293,370). Once the diagnosis of pheochromocytoma has been made based on secretion of catecholamines, localization of the tumor must be performed using computed tomography (CT) or magnetic resonance imaging. These techniques are

especially useful in finding extra-adrenal tumors. Scintigraphy using metaiodobenzylguanidine (MIBG), an analog that resembles norepinephrine, can complement the use of CT adding to specificity of the test (293). Newer compounds such as [^{18}F]-fluoro-2-deoxy-D-glucose combined with positron emission tomography have further added sensitivity particularly in the evaluation of metastatic lesions (293). Genetic testing is recommended for patients with a family history, presentation at less than 50 years of age, or where there are bilateral, malignant, or recurrent tumors (293).

Most pheochromocytomas are benign, but malignant tumors represent 5% to 26% of these neoplasms (375). Benign tumors may be cured by surgical excision, although recurrence has been reported (293). Laparoscopic removal is appropriate for tumors less than 10 cm.

Pathologic Findings

Gross Appearance Pheochromocytomas are located within the medulla of the adrenal gland. Smaller tumors are surrounded by a rim of yellow cortex. The tumor itself is grayish pink. Larger tumors may be hemorrhagic and necrotic. Malignant tumors tend to be larger, contain more necrosis, and invade local tissues. When pheochromocytomas are fixed in formalin and then are exposed to light, they turn a light brown. However, tumors fixed in a bichromate solution turn dark brown.

Microscopic Findings Most pheochromocytomas are composed of large, pink, polygonal cells, often arranged in a trabecular organoid or alveolar pattern within a thin vascular stroma (Fig. 20.40). The typical arrangement forms so-called *Zellballen*. On occasion, the cells are smaller. At other times, the cells are spindled. The tumor is usually surrounded by a thin capsule. Immunohistochemical staining for chromogranin A is strong in pheochromocytomas and distinguishes them from nonneuroendocrine tumors such as those in the adrenal cortex (369). The WHO classification defines malignant pheochromocytomas by the presence of metastases in locations without chromaffin tissues (369). Histologic features such as greater cellular polymorphism and necrosis may be present more frequently in malignant tumors but do not define them. During the last decade, several classification systems have been devised combining histologic, immunochemical, and biochemical characteristics in an attempt to assess malignant potential without real success (369).

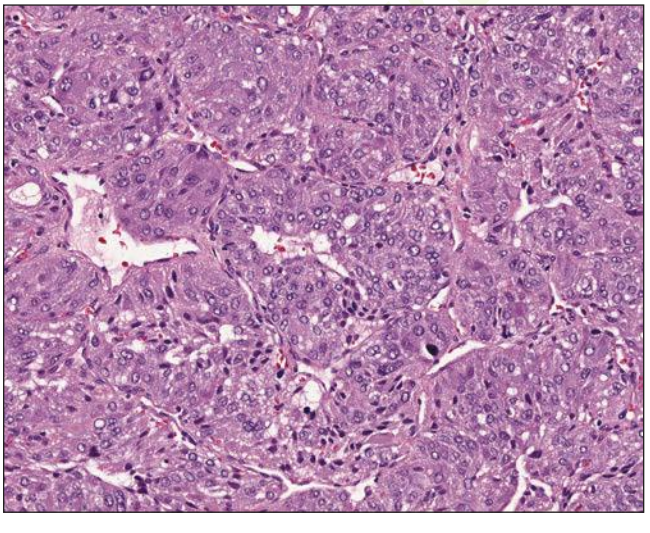

FIGURE 20.40 Pheochromocytoma showing nests of cells in vascular stroma. The tumor cells contain ample granular cytoplasm. (H&E, ×260.)

The ultrastructure of pheochromocytomas is characterized by the presence of dense-core neurosecretory granules with the typical appearance of either epinephrine or norepinephrine granules or sometimes both within the same cell. Immunohistologic studies have shown the presence of chromogranin, neuron-specific enolase, dopamine, ACTH, VIP, calcitonin, and somatostatin.

Conditions Associated With Adrenal Cortical Lesions

Hyperaldosteronism

Hypertension may result from the excess production of aldosterone, as first shown by Conn (376) in relation to removal of an adrenal cortical tumor. Hypertension is typically accompanied by hypokalemia, muscular weakness, decreased plasma renin concentration or activity, and increased plasma aldosterone. However, it is now recognized that many patients with PA may have normokalemia. The current prevalence of PA is 11.2% of hypertensive patients as determined by a prospective study using the aldosterone: renin ratio (377).

The causes of PA are shown in Table 20.5 grouped by those that are surgically curable or not (378–380). Approximately 45% of the cases of PA had surgically correctable hypertension (377). It is important to establish the diagnosis of PA early as such patients are more prone to target organ damage and cardiovascular events than patients with other forms of hypertension (378). Surgical correction by adrenalectomy can avoid such morbidity. Hypertension in most cases of PA is volume dependent with increased blood volume and cardiac output, although increased peripheral resistance eventually becomes predominant.

Numerous mendelian forms of human hypertension associated with hyperaldosteronism and low renin have now been described. These have been reviewed by several authors (21,381,382). They are listed in Table 20.6 and are briefly described below. In glucocorticoid-remediable aldosteronism, a chimeric gene of aldosterone synthase and 11β-hydroxylase is

TABLE 20.5 Causes of primary hyperaldosteronism

Surgically curable
Aldosteronoma
Primary unilateral adrenal hyperplasia
Multinodular unilateral adrenocortical hyperplasia
Ovary aldosterone-secreting tumor
Familial type II hyperaldosteronism
Aldosterone-producing adenoma or bilateral adrenocortical hyperplasia associated with concomitant pheochromocytoma
Aldosterone-producing carcinoma
Not surgically curable
Bilateral adrenocortical hyperplasia
Unilateral aldosterone-producing adenoma with bilateral adrenal hyperplasia
Familial type I hyperaldosteronism (also known as glucocorticoid-remediable hyperaldosteronism)

TABLE 20.6 Inherited forms of primary aldosteronism

Glucocorticoid-remediable aldosteronism (familial hyperaldosteronism type I)
Liddle syndrome
Apparent mineralocorticoid excess
Gordon syndrome
Hypertension exacerbated in pregnancy
Familial hyperaldosteronism type II

expressed in the zona fasciculata under the control of ACTH. The resulting synthesis of aldosterone is not suppressed by AII, and its secretion is unrestrained (382). Liddle syndrome is caused by mutations in the epithelial sodium channel leading to increased numbers of channels at the cell surface. This process results in increased salt and water reabsorption independent of mineralocorticoid levels (21). The syndrome of apparent mineralocorticoid excess is characterized by the deficiency of an isoform of the enzyme 11β-hydroxysteroid dehydrogenase, which catalyzes the interconversion of hormonally active cortisol to inactive cortisone and dictates specificity for the mineralocorticoid receptor (21). This receptor binds aldosterone and cortisol with equal affinity. Normally, this enzyme is expressed in the kidney, with resulting inactivation of cortisol and greater access of aldosterone to the receptor. However, when this enzyme is deficient, more cortisol is bound to the receptor in preference to aldosterone. Cortisol is both more potent and present in greater concentration than is aldosterone, and its binding to the receptor results in an apparent mineralocorticoid excess (21). Gordon syndrome is associated with mutations in either WNK1 or WNK4 kinases leading to increased activity of the thiazide-sensitive sodium chloride cotransporter (383). Hypertension exacerbated in pregnancy results from a missense mutation in the mineralocorticoid receptor that presents with hypertension before age 20 (21). The hypertension is exacerbated during pregnancy because steroids lacking 21-hydroxyl groups, such as progesterone, which is elevated during pregnancy, are agonists of the mutant receptor. A second form of familial hyperaldosteronism that cannot be suppressed by glucocorticoids has been described and several possible candidate genes have been identified (384). These patients have either adrenal cortical hyperplasia or aldosterone-producing tumors.

Screening for PA should be undertaken in hypertensive patients with hypokalemia, resistant hypertension, early onset of hypertension, first-degree relative with PA, incidentally found adrenal tumor, OSA, or target organ damage more severe than expected compared to severity of hypertension (379,380). An algorithm for this screening is provided by Rossi (380). The first step is the determination of the aldosterone: renin ratio with additional tests of exclusion for those with borderline tests. Imaging is then undertaken using high-resolution CT scan. Adrenal vein sampling is necessary to confirm lateralization of increased aldosterone synthesis. Adrenalectomy can then be performed with expectation of cure.

Cushing Syndrome and Related Conditions

Congenital Adrenal Hyperplasia Congenital adrenal hyperplasia (CAH) syndromes are autosomal recessive conditions resulting from a defect in one of the five steps in biosynthesis of cortisol. Deficiency of 21-hydroxylase accounts for more than 90% of the cases (385). Patients may present with hypertension, salt wasting, adrenal insufficiency, or sexual development anomalies. More than 100 mutations of 11β-hydroxylase (*CYP11B1*) have been found that result in a deficiency of 11β-hydroxylase that prevents production of cortisol even in the face of elevation of ACTH (385). Similarly, mutations of 17α-hydroxylase (*CYP17*) result in deficiency of 17α-hydroxylase, which is also critical for the production of cortisol (386). In both cases, the increased ACTH leads to increased deoxycortisol and deoxycorticosterone that produce sodium retention, hypervolemia, and hypertension. Other deficiencies reported in association with CAH include 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase/17, 20-lyase, steroidogenic acute regulatory (StAR) protein (lipoid hyperplasia), and a defect in P450 oxidoreductase (385).

Cushing Syndrome Cushing syndrome is caused by an excess of cortisol. It is manifested clinically by hypertension, truncal obesity, characteristic facies, hirsutism, disturbed glucose tolerance, easy bruisability, striae, poor wound healing, muscle wasting, and osteoporosis (387,388). It may result from the administration of exogenous steroids (including topical preparations and intra-articular injections), from the release of ACTH from pituitary tumors (Cushing disease) or from other tumors (ectopic ACTH), or from synthesis within various tumors of the adrenal cortex (387,388). Guidelines for the diagnosis of Cushing syndrome have been provided from the Endocrine Society (389).

Cushing Disease and Ectopic ACTH Syndrome Cushing disease is found most often in young women, usually in association with a microadenoma of the pituitary gland composed of either basophils or chromophobes (390). Ectopic production of ACTH may be seen in several different tumors, including small cell carcinomas of the lung, carcinoid tumors, islet cell tumors of the pancreas, medullary carcinoma of the thyroid, and pheochromocytoma (388,390). The result of increased serum ACTH is hyperplasia of the adrenal cortex accompanied by increased plasma cortisol and increased excretion of 17-hydroxysteroids and 17-ketosteroids. Pituitary tumors tend to show suppression of ACTH in response to dexamethasone in contrast to ectopic sites of production (390). The transsphenoidal approach is preferred for surgical extirpation (390).

Pathologic Findings

Adrenal Hyperplasia Adrenal nodules are commonly found at autopsy and increase in frequency with age or in patients with hypertension or diabetes (391). Approximately one third of the cases of hyperaldosteronism are due to hyperplasia of the zona glomerulosa (392). Such hyperplasia is characterized by increased width of the cortex and may be accompanied by either large or small nodules. The glands may be normal in weight. By contrast, in Cushing disease, the adrenal glands are often enlarged and show bilateral diffuse cortical hyperplasia with or without nodules (391).

Adrenal Adenoma Grossly, adenomas are yellow, circumscribed, and usually weigh less than 50 g (393). The cells of the tumor have several different appearances. The most common is a large polygonal cell with clearing of the cytoplasm similar to that seen in normal zona fasciculata (Fig. 20.41). The cells are

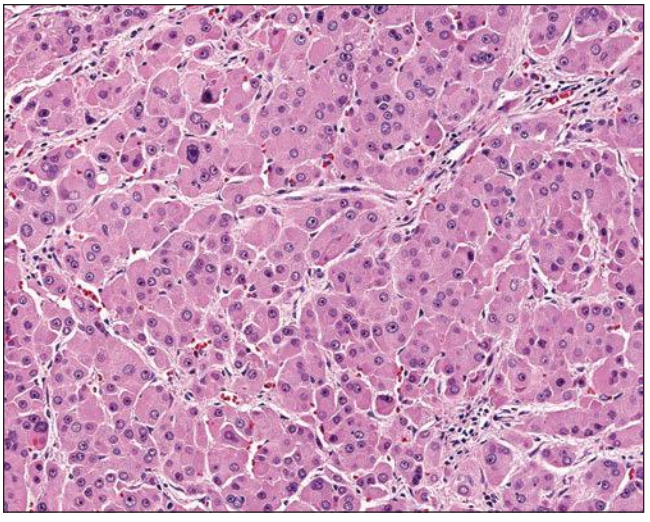

FIGURE 20.41 Adrenal cortical adenoma with large pink cells with ample cytoplasm and vesicular nuclei. An area with nuclear pleomorphism is present in the upper left portion of the micrograph. (H&E, ×260.)

frequently present in alveolar cords or nests. Other cell types include a hybrid form of small, lipid-rich cells resembling a mixture of cells from zona glomerulosa and zona fasciculata, cells resembling those of the zona glomerulosa alone, and some that are similar to those of the zona reticularis. Necrosis and hemorrhage may occur. Ultrastructural studies in tumors that produce aldosterone show the typical cytoplasmic features of cells of the zona glomerulosa (394). These changes include mitochondria with plate-like cristae, smooth endoplasmic reticulum, lipid droplets, polyribosomes, and Golgi complex. In addition, spironolactone bodies may be identified by the concentric, dense lamellae, which resemble myelin figures. High concentrations of aldosterone are present within these tumors (395), but, on occasion, other substances may be detected.

The incidence of adrenal tumors is not known, but they are found incidentally in 4% of patients who undergo high-resolution abdominal imaging studies (396). Most of these are benign. Approximately two thirds of the patients with PA have a small adrenal adenoma, whereas 15% to 20% of patients with Cushing syndrome have an adrenal tumor that is benign in half of the cases (391).

Once an adrenal incidentaloma is discovered, hormonal evaluation for increased production of aldosterone, corticosteroids, or epinephrine/norepinephrine should be undertaken. If the tumor is nonfunctional and less than 3 cm in diameter, it can be monitored safely by repeat radiologic studies (396). If it is functional, it should be removed. Surgical removal of these tumors frequently results in cure, unlike the results of adrenalectomy in cases of pure hyperplasia (392). Predictors of cure include young age, ability to localize aldosterone secretion to one side, and lower plasma renin activity. Expression of CYP11B2, which indicates aldosterone production, in a dominant nodule is also predictive of cure (397).

Adrenal Carcinoma The incidence of adrenal carcinomas is approximately one case per million. (398). In general, adrenal carcinomas are large, usually greater than 6 cm in diameter and may show necrosis grossly (391). Malignancy is determined by assessment of nine features, including eosinophilic cytoplasm, diffuse architecture, necrosis, nuclear atypia, ≥6 mitoses per high-power field, atypical mitoses, and sinusoidal, venous, or capsular invasion (398). Currently, biomarkers have not shown consistent results for determining prognosis (398). Transcriptome analysis has also been applied to adrenocortical tumors, but the results are not yet predictive (399). Carcinomas may produce different steroids at various times during their development (388).

Bartter Syndrome and Other Assorted Tubulopathies

Clinical Features

Bartter syndrome (400) is characterized by hypotension or normal blood pressure despite biochemical and hormonal changes that would suggest that hypertension could be present. Classical Bartter syndrome presents in children with hypokalemia, hyperreninemia, high AII, hyperaldosteronemia, hypercalciuria, and hypokalemic alkalosis. This syndrome and its variants occur in an autosomal recessive pattern and are due to a mutation in any one of six genes involved in salt resorption in the thick ascending limb of Henle (401). The result is marked salt wasting. The increased sodium delivery to the distal tubule results in hypokalemic alkalosis. The faulty sodium chloride reabsorption causes volume depletion activating the RAS (402). Other mutations in genes involved in sodium transport lead to hypertension. Liddle syndrome is due to mutations in the epithelial sodium channel of the cortical collecting duct (403). Gordon syndrome is due to mutations in with-no-lysine kinases, which regulate the function of the sodium chloride cotransporters of the distal convoluted tubule.

Pathologic Findings

The characteristic finding in Bartter syndrome is extensive enlargement of the JGA (Fig. 20.42). In some cases, the macula densa is more prominent, but usually, the entire apparatus is enlarged. Morphometry of the JGA in Bartter syndrome has shown that the JGA may be increased in area by greater than 50% above normal. Immunocytochemical techniques demonstrate increased numbers of cells containing renin, especially in the afferent arterioles (404). Electron microscopy of the JGA in Bartter syndrome shows evidence of increased protein synthesis as manifested by increased rough endoplasmic reticulum with dilated cisterns, abundant free ribosomes, enlarged Golgi apparatus, and granules in different stages of development (404).

Hypertension Associated With Assorted Tumors

Juxtaglomerular Cell Tumors

In 1967, Robertson et al. (405) reported the first case of a juxtaglomerular cell tumor (JGCT), although they described it as a hemangiopericytoma. This tumor contained a pressor substance considered to be renin. The 16-year-old patient presented with malignant hypertension (250/150 mm Hg), papilledema, and low serum potassium. Histologically, the tumor had sheets of large epithelioid cells with prominent vascular spaces. The tumor cells converged into the muscular walls of the arterioles within the substance of the tumor. Granules present in the cells stained with the Bowie stain, a finding indicative of renin.

A recent review of the world's literature showed more than 100 cases of JGCT with presentation most commonly in the second and third decades (406). A female predominance is noted. The presenting symptoms and signs include headache,

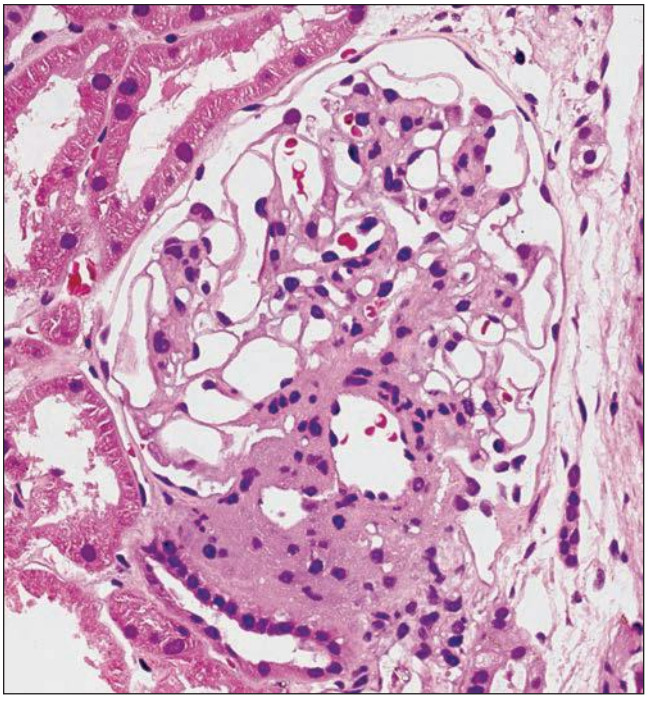

FIGURE 20.42 Hyperplastic juxtaglomerular apparatus (JGA) from a patient with Bartter syndrome. Every JGA in this biopsy showed similar changes. The average JGA shows approximately eight cells and is smaller in area as well. (H&E, ×330.)

severe hypertension, and hypokalemia due to the hyperaldosteronism, proteinuria, dizziness, and retinopathy (406). The possibility of JGCT should be considered in an adolescent or young adult with severe hypertension in combination with unexplained secondary hyperaldosteronism. The renin secretion from these tumors is autonomous such that the hypertension may be difficult to control (407). Surgery is usually curative (407,408).

Grossly, these tumors are usually less than 5 cm in diameter, circumscribed, and gray or yellow white (406,408). They are characterized by sheets of polygonal cells within a vascular stroma. Microcystic structures may be present. Mitoses are rare. At times, the arteries can show intimal thickening and may become hyalinized (406,408) (Fig. 20.43). Electron microscopy demonstrates the typical paracrystalline rhombi seen in the JGA (406,408). Renin has been detected in the tumor cells using both immunohistologic and immunoelectron microscopic techniques (406). In situ hybridization demonstration of renin mRNA has proved that the tumor is synthesizing this product (409). Immunoperoxidase techniques show positivity for actin and CD34 (408). These tumors are considered benign.

Neuroblastoma

Mason et al. (410) reported the first case of hypertension-related neuroblastoma in which blood pressure decreased on removal of the tumor. These tumors can synthesize catecholamines such that some patients present with hypertension (411). Fan (412) presented a series of eight patients with renal neuroblastoma. All eight had hypertension and increased urinary catecholamine.

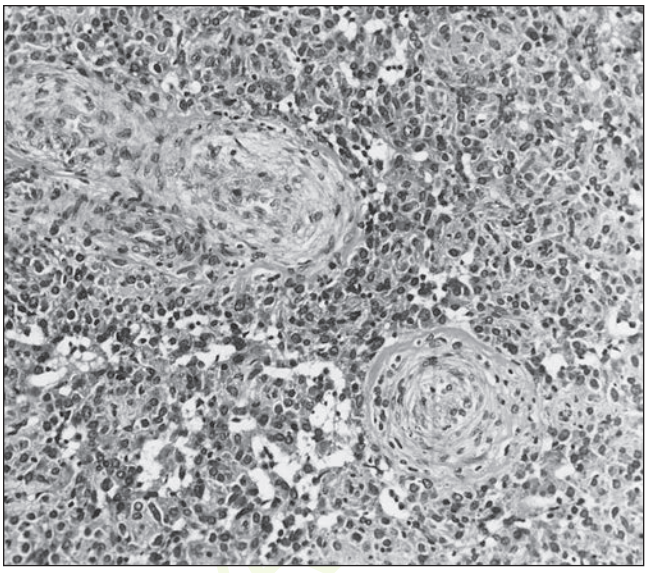

FIGURE 20.43 Renin-secreting tumor made up of polygonal cells. Several small arteries show considerable intimal thickening. (H&E, ×144.) (Courtesy of Dr. A. M. Jackson.)

Nephroblastoma (Wilms Tumor)

The first report of an association between hypertension and nephroblastoma was published in 1937 (413). Hypertension in patients with nephroblastoma is fairly common and is usually secondary to intrarenal ischemia due to compression on the renal vessels by the tumor (414). Lindop et al. (415) found renin production with elevated plasma renin activity in 10 of 19 nephroblastomas. Usually, this form of renin was inactive. Immunohistochemical examination revealed renin in ovoid cells near tumor vessels in areas of well-differentiated mesenchyme, a finding suggesting that the tumor cells themselves produced the renin. D'Angelo et al. (416) described two children who presented with hypertension, hyponatremia, and an abdominal mass. One case showed compromise of renal blood flow by the Wilms tumor. Immunohistochemistry demonstrated renin in atrophic glomerular structures in both cases. Nephrectomy cured the hypertension in both the cases.

Renal Cell Carcinoma

Numerous epidemiologic studies have shown that hypertension is a risk factor for renal cell carcinoma (417,418). In some reports, reduction in blood pressure followed nephrectomy (419). Steffens et al. (420) studied 129 patients with renal cell carcinoma. Forty-one had hypertension; however, resection of the tumors led to normalization of blood pressure in only six of these patients. These six patients also had high plasma renin activity in the ipsilateral renal vein and higher renin levels in the tumor tissue than in the remaining kidney. Furthermore, no evidence of compression of the renal arteries was seen in any of these six patients. These investigators suggested 3 possible mechanisms of hypertension in the 41 patients with elevation of blood pressure. These were tumor compression of the renal artery, arteriovenous fistula within the tumor-producing ischemia of renal tissue, or, as found in the foregoing six patients, autonomous renin production by the tumor itself. Histochemical studies suggested that renin

production occurred in endothelial cells within the tumor. No staining for renin was seen in the other 123 cases. Where possible, the current practice is to perform partial nephrectomy in order to spare renal parenchyma. It has been recently suggested that this practice has been underutilized particularly in patients with diabetes and hypertension (421).

Miscellaneous Tumors

Various other tumors have also been reported to be associated with hypertension chiefly through the production of renin (407,409). Other mechanisms for the association between tumors and hypertension have also been described. As noted in the previous paragraph, large renal cell carcinomas may compress the renal artery and may produce stenosis. Tsuchida et al. (422) reported a case of congenital mesoblastic nephroma in a 39-day-old boy with elevated plasma renin activity and total renin concentration. His mild hypertension returned to normal after extirpation of the tumor. Immunoperoxidase studies showed renin in the JGA of glomeruli trapped within the tumor, rather than in the tumor cells. Neilly et al. (423) described two patients with non-Hodgkin lymphoma with hypertension thought to be secondary to a lymphomatous infiltrate in the kidney that produced ischemia. The blood pressure returned to normal with successful treatment, as manifested by reduction in kidney size.

Angiolymphoid Hyperplasia with Eosinophilia

The condition of angiolymphoid hyperplasia with eosinophilia is characterized by subcutaneous nodules with vessels that have enlarged and proliferating endothelial cells with an inflammatory infiltrate composed of lymphocytes, lymphoid follicles, and eosinophils. Fernandez et al. (424) described a case in which excision of two such nodules was associated with reduction of hypertension. Renin was identified in cells surrounding the vessels. Six of eight other cases also showed renin-containing cells.

Obstructive Sleep Apnea

The association between OSA and hypertension has been recognized for years. The prevalence of hypertension in OSA patients with daytime symptoms is 35% to 80% increasing with more severe degrees of apnea. Conversely, 40% of patients with hypertension had OSA (425). Originally, it was thought that this association could be entirely attributed to obesity. However, OSA has recently been found to be an independent risk factor for hypertension (425). Potential mechanisms include increased sympathetic activity during apneic episodes, endothelial dysfunction, and activation of ROS (425). A recent study compared endothelial function in a group with OSA and hypertension, OSA without hypertension, hypertensive patients without OSA, and a control group without hypertension or OSA (426). They found that the patients with OSA and hypertension had the greatest impairment of flow-mediated vasodilation. Other potential mechanisms such as increased aldosterone, metabolic factors, and possible genetic associations require additional studies.

Oral Contraceptive Agents

The recognition that OCs could induce hypertension in women was made in 1967 by Laragh et al. (427). Stopping administration of the birth control pill reduced blood pressure, and reintroduction raised blood pressure once again. The degree of blood pressure elevation or the frequency of its appearance in association with OC has decreased with the introduction of newer formulations (428). We are currently using the fourth generation with estradiol only 20% of that used in the first generation and with lower doses of progestins that have fewer androgenic and metabolic effects. Some patients become hypertensive with the newer types of OC although not with formulations that contain only the newer progestins (429). OC continue to pose a risk for uncontrolled elevations in blood pressure in patients with previous history of hypertension (430). Stopping OCs in hypertensive patients lowered the systolic blood pressure by 15.1 mm Hg and the diastolic pressure by 10.4 mm Hg (431). A recent study in Chinese women showed that a polymorphism in the estrogen receptor beta acted synergistically with OC to increase the likelihood of hypertension as compared to women taking OC who did not have the polymorphism (432).

Several possible mechanisms have been suggested for the hypertension induced by OCs. The two most widely held include effects on the RAS and on the insulin resistance syndrome (428,433). In the first of these, it is well known that estrogens increase the synthesis of the renin substrate in the liver, a change that may result in mild hyperaldosteronemia. This condition leads to sodium retention. The insulin resistance syndrome is characterized by hyperinsulinemia, impaired glucose tolerance, hypertriglyceridemia, reduced high-density lipoprotein, and hypertension (434). OCs have also been shown to increase arterial stiffness (435). These disorders can all be induced by various formulations of OCs.

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CHAPTER 21

Jean L. Olson • Zoltan G. Laszik

Diabetic Nephropathy

DIABETIC NEPHROPATHY

Diabetes mellitus (DM) is recognized in two forms, classified as type 1 and type 2 DM (1). Type 1 DM includes those cases that are primarily due to pancreatic β-cell destruction. The form named type 2 DM includes the most prevalent form of the disease, which results from insulin resistance with an insulin secretory defect. Type 1 DM accounts for 5% to 10% of people with DM, and type 2 DM affects approximately 90% of those with the disease (2). The prevalence of DM worldwide was estimated to be 2.8% in 2000 and is projected to increase to 4.4% by 2030 (3). The natural history, pathologic features, and pathogenesis of diabetic nephropathy are remarkably similar but not identical in patients with type 1 and type 2 DM. Therefore, throughout the chapter, we comment on these variations, when appropriate.

Diabetic nephropathy is a clinical syndrome characterized by persistent albuminuria (greater than 300 mg/24 hours) (also referred to as macroalbuminuria or proteinuria), a steady decline in glomerular filtration rate (GFR), and elevated blood pressure (4). Two thirds of patients with diabetic nephropathy develop renal failure requiring either dialysis or renal transplantation. Diabetic nephropathy is the most common cause of chronic renal failure in the United States and accounts for 43.9% of patients enrolled in long-term dialysis programs (5). Patients with nephropathy frequently develop other complications, in particular cardiovascular disease, including hypertension and stroke, resulting in increased risk of early mortality (6,7). In patients with type 1 DM, 40 years after onset of the disease, the mortality rate is 90% for those patients with nephropathy but only 30% for those patients without renal disease (7). End-stage renal disease (ESRD) in type 1 diabetics results in a 13-fold increase in the risk of death (8). Thus, renal complications of DM are important, and glomerulosclerosis and vascular disease are the most important causes of renal failure in the diabetic patient.

Frequency and Risk Factors

Incidence and Prevalence

Several studies from the 1980s showed a cumulative incidence of diabetic nephropathy of 25% to 40% after 25 years of type 1 DM with a declining trend in cumulative incidence compared to those patients diagnosed in the 1930s and in the 1950s (9–11). A study from Sweden published in 1994 reported a dramatic decline in the cumulative incidence of diabetic nephropathy in patients diagnosed before age 15 years (12). The investigators showed that the cumulative incidence of diabetic nephropathy after 25 years declined from 28% in those patients with an onset of type 1 DM between 1961 and 1965 compared with 8.9% in those patients with an onset between 1966 and 1970 and 5.8% in a cohort from 1971 to 1975. Most follow-up studies have confirmed the declining trend of incidence of diabetic nephropathy in patients with type 1 DM thought to be secondary to better control of hyperglycemia (13–15).

Older data showed a cumulative incidence of ESRD of 2% to 8% in patients with type 1 DM 20 years after the onset of DM and 15% to 17% after 30 years (16). A more recent study from Finland shows a decline in cumulative incidence of ESRD to 7.8% after 30 years (8). The peak annual incidence of developing proteinuria in type 1 DM is approximately 3% between 10 and 20 years of DM duration (9,17).

The prevalence of proteinuria in patients with type 1 DM is between 15% and 40% (9,13).

The prevalence and cumulative incidence of proteinuria (as a measure of diabetic nephropathy) in patients with type 2 DM are comparable but not identical with those of type 1 DM (18–20). Adler et al. (18) found that progression to microalbuminuria occurred at a rate of 2% per year from the diagnosis of type 2 DM with progression to macroalbuminuria from microalbuminuria occurring at a rate of 2.8% per year. The prevalence of microalbuminuria at 10 years was 24.9% and for macroalbuminuria was 5.3% in these patients in the United Kingdom. A more recent study in the United Kingdom has demonstrated a prevalence of chronic kidney disease (CKD) stages 3 to 5 ranging between 18% and 27.5% at 10 years after diagnosis (15). In contrast, the prevalence of macroalbuminuria in a study from India was 44% with a median follow-up of 11 years (20).

Duration of Disease

The incidence of diabetic glomerulosclerosis increases with the duration of DM. This correlation was shown most clearly by Andersen et al. (9) in a large cohort study from Steno Memorial Hospital. These authors studied 1475 patients with type 1 DM for ≥25 years or until death and found an increase in the annual incidence of renal disease through the first 16 years after diagnosis of DM with a decline in the number of patients developing renal lesions after that period. Only 4% of patients developed nephropathy after 35 years of DM. These findings have been confirmed in a study of more than 20,000 patients from Finland (8). Overt nephropathy caused by glomerulosclerosis first appears 10 to 15 years after the onset of type 1 DM and after 5 to 10 years in patients with type 2 DM (21). While diabetic nephropathy is rare before 10 years of DM duration in patients with type 1 DM, approximately 3% of patients with newly diagnosed type 2 DM have evidence of diabetic nephropathy (22). The biologic onset and duration of type 2 DM are often uncertain so that the course of diabetic nephropathy in this patient population is less well defined than in patients with type 1 DM. In addition, the more frequent comorbidities in patients with type 2 DM, such as hypertension and cardiovascular diseases, may alter the clinical presentation of nephropathy in patients with type 2 DM.

Gender

Various studies show either male or female predominance in the incidence of DM (5,8). However, the incidence of diabetic renal disease has shown a predominance of males compared with females (5,7).

Racial and Ethnic Factors

Several distinct racial and ethnic groups have a greater incidence of type 2 DM than do other such groups. Nephropathy is also more common in the diabetic populations of various ethnic and racial groups. The prevalence of diabetic nephropathy is highest in Native Americans, followed by African Americans, Hispanics, and Caucasians (23–25). The Pima Indians of the Southwestern United States have the highest prevalence of type 2 DM (26). Fully half of all Pimas develop type 2 DM by the time they have reached 35 years of age (26). African Americans and Hispanic Americans also have a higher incidence both of DM and of the associated diabetic nephropathy (23,27). These differences in prevalence of diabetic nephropathy among ethnic groups are present after adjustment for other risk factors such as hypertension, socioeconomic status, age, and prevalence of DM within the population (27). In a study of patients with type 2 DM, de Zeeuw et al. (28) found a slightly increased risk for ESRD in Asians and Hispanics relative to the black and white populations examined. Multiple regression analysis demonstrated that baseline albuminuria was the best independent predictor of ESRD in all ethnicities studied.

Genetic Factors

Several lines of evidence support an important role for various genetic factors in the development of diabetic nephropathy. The fact that diabetic nephropathy develops only in a subset of patients with DM has long been interpreted as evidence pointing to genetic factors in its pathogenesis. Familial clustering of diabetic nephropathy in both type 1 and type 2 DM is another indication for probable genetic predisposition for the disease (17,29,30). Evidence for genetic predisposition for diabetic nephropathy is also present in patients with type 2 DM. Sib-pair linkage analysis in Pima Indians with type 2 DM identified strong linkage for diabetic nephropathy on chromosome 7q and also on chromosomes 3, 9, and 20 (31).

Three different approaches may be taken to identify potential genes. These include candidate genes, linkage analysis, and genome-wide association scans (GWAS) (32,33). The candidate gene approach assesses genetic variation by examining single nucleotide polymorphisms (SNPs) in genes that have a possible role in the pathogenesis of the trait being studied. Linkage analysis involves interrogation of hundreds of markers, usually microsatellite markers, which are spread evenly across the genome to identify regions that are coinherited with the trait of interest. Linkage analysis can be further refined in genome scan meta-analysis in which linkage data from independent studies are synthesized. GWAS analyzes large numbers of markers, usually SNPs, in DNA samples from many individuals in order to detect common genetic variations associated with the disease of interest. Results from these various forms of analysis will be considered in turn.

The candidate gene approach studies SNPs in genes involved in metabolic pathways that contribute to the pathogenesis of the changes seen in diabetic nephropathy. Examples for such candidate genes include those (a) mediating the synthesis and degradation of the glomerular capillary basement membrane and mesangial matrix components; (b) components of blood pressure regulation and the renin-angiotensin system (RAS); (c) various cytokines, growth factors, signaling molecules, and transcription factors; (d) advanced glycation processes; (e) activation of protein kinase C (PKC) isoforms; (f) formation of reactive oxygen species (ROS); (g) increased activity of the aldose reductase (AR) pathway; and (h) genes mediating glucose metabolism and transport (33). Angiotensin-converting enzyme (ACE) plays an important role in the regulation of blood pressure and has a well-known insertion (I)/deletion (D) polymorphism that has been studied in many renal diseases. Persons with the II genotype have the lowest ACE concentrations, while those with the D allele have increased ACE levels (34). A recent large meta-analysis compared the I/D genotype of 26,580 persons with or without diabetic nephropathy and found an increased risk for that condition in the Asian subset with type 2 DM and the presence

of the D allele (35). A similar increased risk for ESRD due to diabetic nephropathy was found in a second meta-analysis in Asian patients with type 2 DM and the DD genotype (36). An early study analyzed 115 candidate genes using a transmission/disequilibrium test in patients with type 1 DM and identified 20 genes with polymorphism (37). However, in spite of a large number of studies that tested the association of candidate genes with diabetic nephropathy, only three analyses have found convincing candidate genes with replicate studies, including PKC–β (38), erythropoietin gene promoter (39), and endothelial nitric oxide synthase (40). However, other investigators (41) using a large case-control meta-analysis were unable to confirm an association of the erythropoietin gene promoter as well as several other previously published associations to diabetic nephropathy.

The first linkage study was performed in Pima Indians and showed linkage of loci on chromosome 7 to diabetic nephropathy (31). Linkage studies in a Turkish kindred with diabetic nephropathy found a linkage peak on chromosome 18 (42). Later studies found a candidate gene in this locus, namely, carnosinase-1 (*CNDP1*) (43). Carnosine acts as an oxygen free radical scavenger and may interfere with the formation of advanced glycosylation end products (32). The Family Investigation of Nephropathy in Diabetes (FIND) study was a genome-wide linkage study of sibling pairs concordant or discordant for diabetic nephropathy in several different ethnic groups (44). This study then spawned additional analyses that confirmed earlier linkage peaks in various subpopulations (32,45–47). In general, these family-based linkage studies have lacked consistency and have not succeeded in identifying genetic loci in complex traits such as diabetic nephropathy.

In GWAS, the genome is scanned for SNPs in order to detect common variations for a particular disease. The technology has advanced so that it is now possible to scan up to 5 million SNPs (32). Once possible susceptibility loci are found, they must be replicated in other databases. The first GWAS on diabetic nephropathy was performed on a Japanese population and identified the engulfment and cell motility 1 gene (*ELMO1*) (48). A pooling approach was used in a GWAS of Pima Indians that found an association between the plasma cell variant translocation 1 locus and diabetic nephropathy (49). Several GWAS have been undertaken using the Genetics of Kidney in Diabetes (GoKinD) collection and have identified possible susceptibility loci (50,51). Additional studies in other ethnic groups have also found susceptibility loci (50,52–54). Selected loci are shown in Table 21.1 (50,55). Several investigators have performed meta-analyses of GWAS studies or have used imputation of SNPs in an attempt to find additional susceptibility loci (41,51,56). Sandholm et al. (56) found two SNPs associated with ESRD in type 1 diabetics with diabetic nephropathy. These SNPs were located in the *AFF1* gene, which is involved in the transforming growth factor–β pathway and in an intergenic region on chromosome 15q26. These authors also demonstrated an association between diabetic nephropathy and an intronic SNP in the *ERBB4* gene (56). Some studies using meta-analysis have failed to confirm previous genes (41). Bowden and Freedman (57) have suggested several explanations for seeming differences including the possibility that a particular gene may be important in one ethnic group and not in another. These studies are highly technical and currently provide locations of SNPs associated with either type 1 or type 2 diabetic nephropathy. Additional work will be required to determine the relevance of these SNPs to the pathogenesis of diabetic nephropathy.

TABLE 21.1 Selected possible susceptibility loci for diabetic nephropathy

Nearest gene(s)	Chromosome	Risk allele	Odds ratio
FRMD3	9	A	1.5
CARS	11	A	1.4
PLEKHH2	2	CT	1.4
MSC2	8	G	1.6
ZIMZ1	10	G	1.5
PVT1	8	G	3.0
SLC12A3	16	G	2.5
ELMO1	7	GG	2.7
CPVL/CHN2	7	GA	1.39–1.43

From Pezzolesi MG, Poznik GD, Mychaleckyj JC, et al. Genome-wide association scan for diabetic nephropathy susceptibility genes in type 1 diabetes. *Diabetes* 2009;58(6):1403.

From Drawz PE, Sedor JR. The genetics of common kidney disease: a pathway toward clinical relevance. *Nat Rev Nephrol* 2011;7(8):458.

Epigenetic mechanisms are also now recognized as important risk factors in diabetic nephropathy. Epigenetics are heritable modifications that occur without changing the DNA structure (58). The epigenetic mechanisms that effect these modifications include DNA methylation, histone post-translational modifications, small noncoding microRNAs, and long noncoding RNAs (58). Such mechanisms can affect transcriptional memory and regulate aspects of gene expression, including cellular response to environmental cues. The observation that leads to the recognition of a possible role for epigenetics in diabetic nephropathy was the identification of metabolic memory in clinical trials exploring the efficacy of strict glycemic control in modifying the course of this complication in patients with type 1 DM (59). Numerous such trials (reviewed by Tonna et al. (59)) demonstrated that the beneficial effects of strict metabolic control continued for years after the study period despite return of patients to conventional therapy. Benefits included reduced incidence of new cases of microalbuminuria, reduced risk of progression of renal disease, and a lower mean serum creatinine concentration in the intensive treatment group compared to the conventional treatment group. Similar effects have been observed in numerous studies in patients with type 2 DM (59). In one such study, patients were maintained for a period of 10 years on intensive control or conventional therapy and then returned to conventional therapy and followed for an additional 10 years (60). Despite similarities in hemoglobin A1c within 1 year of return to conventional therapy, the group with intensive therapy showed reduced risk for diabetic retinopathy and/or nephropathy of 25%, myocardial infarction of 15%, diabetes-related death of 17%, and any cause of death of 13% (60). The possible epigenetic mechanisms that explain this metabolic memory have not yet been elucidated. However, it has been shown that epigenetic histone posttranslational modifications may affect expression of nuclear factor (NF)-κB in endothelial cells and monocytes in hyperglycemic conditions (58). Additional evidence for a role for epigenetic factors in diabetic nephropathy is

provided in a study in which an inhibitor of histone deacetylase was administered to rats with streptozotocin (STZ)-induced DM (61). Kidney growth is one of the first changes seen in this model. The inhibitor reduced epithelial growth factor receptor mRNA and protein, resulting in decreased tubular proliferation. As we learn more about the epigenome, we will be able to determine the role of particular mechanisms.

Smoking

Cigarette smoking is a well-established independent risk factor for the development of the microvascular complications of DM. There are also data indicating that cigarette smoking contributes to the development of diabetic nephropathy. Cigarette smoking increases urinary albumin excretion (UAE) in patients with type 1 DM and also in type 2 DM (62,63). In addition, smoking cessation is associated with reduced UAE in patients with type 1 DM (64). Hua et al. (65) showed that nicotine exposure worsened the progression of diabetic nephropathy in mice. It is likely that smoking hastens progression via increase in oxidative stress (66,67).

Clinical Presentation

Clinical Stages

Mogensen et al. (68) described five clinical stages in the evolution of diabetic glomerulosclerosis in patients with type 1 DM. Stage I is characterized by the presence of both hyperfunction, as manifested by increased GFR, and hypertrophy, as recognized by enlargement of the kidneys affecting both glomeruli and tubules. Stage II develops silently over many years in some patients and is marked by the evolution of glomerular lesions in the absence of clinical evidence of renal disease. A correlation exists between elevation of glycosylated hemoglobin and increased GFR at this stage (69). Stage III marks incipient diabetic nephropathy. It is recognized by the occurrence of microalbuminuria, which is defined as ranging between 20 and 200 μg/min (30 to 300 mg/d) on repeated measurements (68). Renal function is preserved as determined by blood urea nitrogen (BUN) and serum creatinine. Increased blood pressure may be present at this stage, but it rarely occurs in patients with UAE less than 100 mg/d (70). Stage IV is characterized by the presence of overt diabetic nephropathy as clinically manifested by proteinuria greater than 300 mg/d and a declining GFR. Microscopic hematuria may be identified in 28% to 48% of patients with diabetic nephropathy (69). This stage is usually associated with rising systemic blood pressure and the presence of other diabetic complications. Development of overt nephropathy with albuminuria was once considered to be the "point of no return" with steady progression to ESRD. However, several studies in patients with type 1 DM have documented beneficial effects of glycemic control and antihypertensive treatment on the progression of diabetic nephropathy (71–73). Stage V is the appearance of ESRD in patients with diabetic nephropathy. More recent studies have demonstrated that the progression of renal disease through these stages may not be inexorable, but that microalbuminuria may regress as discussed in the next section (74). Some authors now suggest using the stages of CKD as defined by the National Kidney Foundation rather than the stages described above (15,75).

The clinical progression to diabetic glomerulosclerosis is not so clear-cut in type 2 DM, partly because of the difficulty of determination of the actual biologic onset of the diabetes itself. Stages I and II in patients with type 2 DM are similar to those seen in patients with type 1 DM (76). It is more difficult to determine the onset of stage III in a patient with type 2 DM. Microalbuminuria, the cardinal laboratory feature of stage III diabetic nephropathy, may be present at the time of diagnosis of DM in as many as 26% of patients (77); however, the microalbuminuria may reflect hypertension, nephrosclerosis, or other related conditions rather than diabetic renal disease.

Microalbuminuria and Risk for Diabetic Nephropathy

Approximately 70% of screened patients with type 1 and type 2 DM have normoalbuminuria (78). Microalbuminuria, defined as 30 to 299 mg/24 hours, a cutoff value adopted by the American Diabetes Association (79,80), has been widely regarded as the best available marker for risk of later development of diabetic nephropathy in both type 1 and type 2 diabetic patients (81–84). However, it is now well known that microalbuminuria does not indicate certain progression to diabetic nephropathy (74,85,86). Microalbuminuria is considered to be a promoter of renal functional deterioration, and a reduction of microalbuminuria or prevention of development of microalbuminuria is important in preserving renal function.

According to several studies, the prevalence of microalbuminuria is below 20% in patients with type 1 DM and, on average, slightly above 20% in patients with type 2 DM (22,26,68,82,87,88). The cumulative incidence of microalbuminuria in patients with type 1 DM was 12.6% over 7.3 years in the European Diabetes Prospective Complications Study Group (89) and approximately 33% in an 18-year follow-up study from Denmark (90). The rate of progression from microalbuminuria to proteinuria over 5 to 10 years in patients with type 1 DM is approximately 15% to 30% (86,91). The estimated incidence of progression from normoalbuminuria to microalbuminuria and subsequent proteinuria during a follow-up of 6 to 9 years in patients with type 2 DM is approximately 20% to 30% (91). A European study using data from greater than 5000 patients with type 2 DM found that progression from normoalbuminuria to microalbuminuria occurred at a rate of 2.0% per year and from microalbuminuria to proteinuria at 2.8% per year (18). It should be noted that such progression is not inevitable. Perkins et al. (74) studied 386 patients with type 1 DM and microalbuminuria persistent for 2 years with follow-up of 6 years. The cumulative incidence of increase to proteinuria was 19% over the 6 years of the study. Regression defined as halving of the microalbuminuria was frequent with a cumulative incidence of 59% (74). Independent predictors of regression included microalbuminuria for shorter interval, hemoglobin A1c less than 8%, systolic blood pressure less than 115 mm Hg, cholesterol less than 198 mg/dL, and triglyceride less than 145 mg/dL. The authors suggested that the microalbuminuria may indicate possible reversible forms of injury. Other investigators have also demonstrated the occurrence of regression of microalbuminuria. In a large European study, a regression rate of 50.6% has been demonstrated in type 1 diabetics with microalbuminuria after 7 years of follow-up (86). In the Steno-2 study, 46 (31%) out of 151 patients with type 2 DM and microalbuminuria reverted to normoalbuminuria during 7.8 years of follow-up (85). In the latter study, antihypertensive therapy and improved glycemic control were independent predictors for remission (85).

Additional studies examined other aspects of the relationship between microalbuminuria and the onset of diabetic nephropathy. In the first of these, Perkins et al. (92) studied 301 diabetic patients with normoalbuminuria and 267 patients with microalbuminuria over a period of 8 to 12 years. Using cystatin C as a measure of GFR, they defined early decline in renal function as a decrease of 3.3% per year. They found such decline in 9% of patients with normoalbuminuria, 16% of those with regression of microalbuminuria, 32% of those with stable microalbuminuria, and 68% of those with progression of microalbuminuria. Thus, microalbuminuria was associated with greater risk of renal decline. However, renal decline also occurred in the absence of microalbuminuria. The authors suggested that GFR should be followed in addition to albuminuria in patients with DM (92). This same group then examined 79 patients with type 1 DM with new onset of microalbuminuria over a period of 12 years (93). Twenty-three progressed to significant chronic renal disease (NKF stages 3, 4, or 5) during the interval of the study, but only 12 had proteinuria. The other 11 in this group had persistent microalbuminuria or even returned to normoalbuminuria. The risk of developing CKD was increased in the presence of proteinuria and elevated glycosylated hemoglobin, but proteinuria was not necessary. It should be noted that biopsies were not performed to determine the type of renal disease present. From this group of studies, the authors conclude that microalbuminuria does increase the risk of developing renal disease in diabetic patients, but it is insufficient to predict diabetic nephropathy or renal function decline. The latter may occur in the absence of proteinuria. Thus, they support the discovery of other biomarkers to aid in such prediction rather than continued reliance on microalbuminuria alone (93).

Biomarkers Other Than Microalbuminuria

With the recognition that microalbuminuria is not sufficient as a biomarker for the presence of diabetic nephropathy, a search for other possible biomarkers began. The first molecules considered were indicators of tubular injury such as neutrophil gelatinase–associated lipocalin (N-GAL) and kidney injury molecule-1 (KIM-1) (94). However, neither N-GAL nor KIM-1 has been shown to be a specific predictor for diabetic renal disease as adjustments for proteinuria negated associations that had been present (94). A third marker of tubulointerstitial injury, urinary liver-type fatty acid–binding protein (u-LFABP), is increased in diabetic patients prior to the onset of proteinuria (95). In this study of 165 patients with type 1 DM, the urinary levels of LFABP predicted the progression of diabetic nephropathy and all-cause mortality independent of UAE and other risk factors (95). However, additional studies are required to validate this result (94).

Investigators at the Joslin Clinic followed 410 patients with DM type 2 for 12 years having measured markers of systemic inflammation, endothelial dysfunction, and the tumor necrosis factor (TNF) pathway at entry (96). They found that circulating TNF receptors 1 and 2 predicted the occurrence of ESRD in this population. These were the only markers to associate with the risk for ESRD. TNFR1 surpassed all other clinical variables examined in the prediction of ESRD (96). In a companion article, the same group found that TNFR1 and TNFR2 predicted declines in renal function in patients with type 1 DM (97). Urinary levels of inflammatory cytokines have also been measured and may prove promising as biomarkers (94).

Urinary proteome analysis is a new technique that may allow detection of patients with diabetic nephropathy (98). In this study, the global protein content of 165 patients was determined by surface-enhanced laser desorption/ionization-time of flight/mass spectrometry and examined by classification and regression tree analysis. The study included normal controls and diabetic patients with normoalbuminuria, microalbuminuria, and biopsy-proven diabetic nephropathy or nondiabetic CKD. In addition, nondiabetic patients with biopsy-proven CKD were included. A specific proteomic signature identified patients with type 2 DM who had diabetic renal disease (98). The two most prominent predictive peaks in this analysis were identified as β2-microglobulin and ubiquitin (98). Of particular interest, it was noted that this analysis could differentiate between diabetic nephropathy and nondiabetic renal disease in diabetics. This technique has also been used by others, and various peptides and orosomucoids have been detected as potential biomarkers for diabetic renal disease (94).

Other potential molecules for study include extracellular matrix (ECM)-related proteins, oxidative stress markers, enzymes, advanced glycation end products (AGEs), podocyte markers, and pigment endothelium–derived factor (94). Validation of any of these potential markers will be required prior to use in the clinical setting. Moreover, as diabetic nephropathy is a complex disease with a multigenetic background as well as multifactorial pathogenesis, it is not likely that a single biomarker will be sufficient to predict disease or progression of the disease.

Pathologic Findings

The possible association of specific glomerular lesions with DM was first recognized by Kimmelstiel and Wilson in 1936 (99). They described a series of eight patients who, at autopsy, had a striking formation of nodules. Seven of these patients had diabetes, whereas the remaining patient was moribund without available clinical history. Edema and heavy proteinuria were present, accompanied by high blood pressure in some patients. At the same time, Murakami (100) in Japan described a similar histologic picture in a single patient. Additional studies since that time have described the histologic alterations that are characteristic of diabetic glomerulosclerosis and are accompanied by a stereotypical clinical course.

Gross Appearance

The kidney of the diabetic patient may be increased, decreased, or normal in size. At early stages, it is invariably increased, particularly in patients with hyperfiltration (68). When diabetic glomerulosclerosis progresses with scarring and loss of nephrons, one sees a reduction in the size of the kidney, but the end-stage kidney of the diabetic patient is frequently larger than the end-stage kidney seen in other conditions (Fig. 21.1). On the cut surface, preservation of normal architecture is noted. With proper lighting, one may be able to detect hypertrophied glomeruli within the cortex. Frequently, the arteries at the corticomedullary junction are prominent because thickening of their walls resulting from arteriosclerosis prevents their retraction into the parenchyma. The main renal artery and its branches may show atherosclerosis.

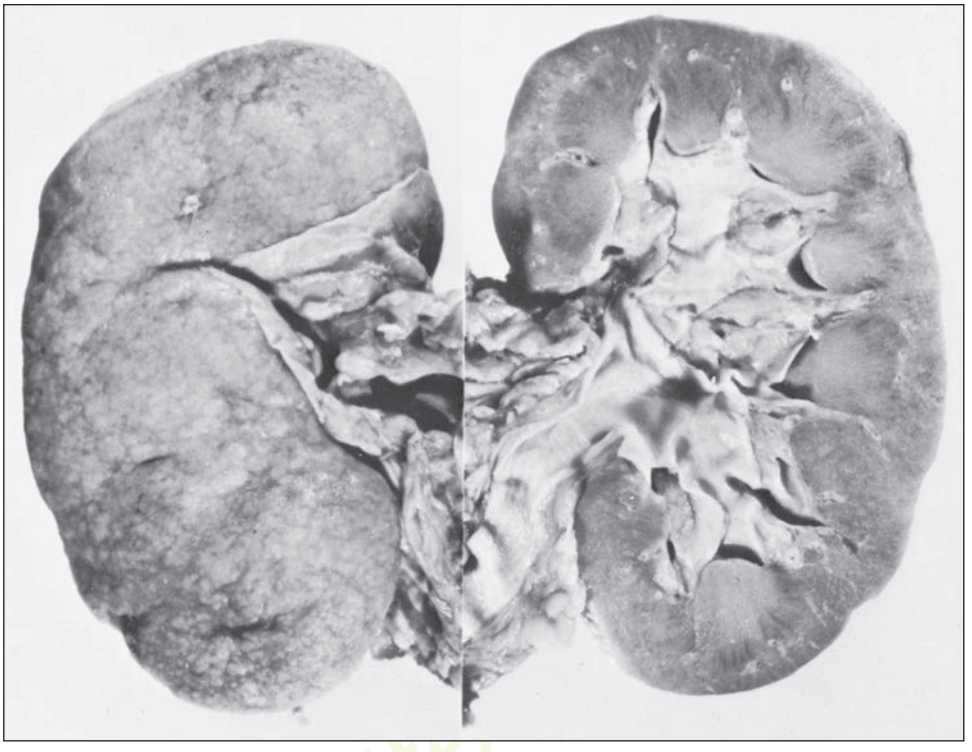


FIGURE 21.1 Kidney from a 48-year-old woman with diabetes of 13 years' duration. Nodular glomerulosclerosis was diagnosed on biopsy after nephrotic syndrome 3 years before death from renal failure. Each kidney weighed 90 g. The subcapsular surface is granular, and the cut surface shows a slight reduction in cortical width.

Light Microscopy

Glomeruli

The glomeruli show a constellation of findings. The earliest change is diffuse uniform thickening of the capillary wall (Fig. 21.2) that may be difficult to detect on light microscopy. Additional alterations include a diffuse form with mesangial sclerosis, manifest as an increase in mesangial matrix, a nodular mesangial lesion sometimes combined with microaneurysms, exudative or hyalinosis lesions, and the capsular drop. At early stages, glomerular volume is enlarged 70% as compared with the glomeruli of nondiabetic subjects (101). Filtration surface area is increased by 80% in a similar comparison.

Diffuse Lesion The diffuse lesion consists of widespread increase in eosinophilic, periodic acid-Schiff (PAS)–positive material within the mesangium as first described by Spuhler and Zollinger (102). Hypercellularity is uncommon (103), although it may occur. Uniform increase in the thickness of the capillary walls may be seen (see Fig. 21.2) as early as 2 years after the onset of DM and may not be accompanied by clinical evidence of renal dysfunction (104,105). The mesangial alterations progress in severity (Fig. 21.3) usually but not always accompanied by concomitant capillary wall thickening, increasing with duration of disease (106). The combination of mesangial expansion and thickened glomerular basement membrane (GBM) results in decreasing the patency of the capillary lumina and decreased filtration surface area. Two populations of obsolete glomeruli are present in diabetic nephropathy. The most prevalent are enlarged solidified glomeruli due to the increase in mesangial matrix (Fig. 21.4). The other form is that typical of ischemic change without increased matrix and showing wrinkling of capillary loops (Fig. 21.5). This latter population is present due to the fact that arteriosclerosis contributes to the evolution of diabetic renal disease. The Bowman capsule may be thickened in the more advanced lesions.

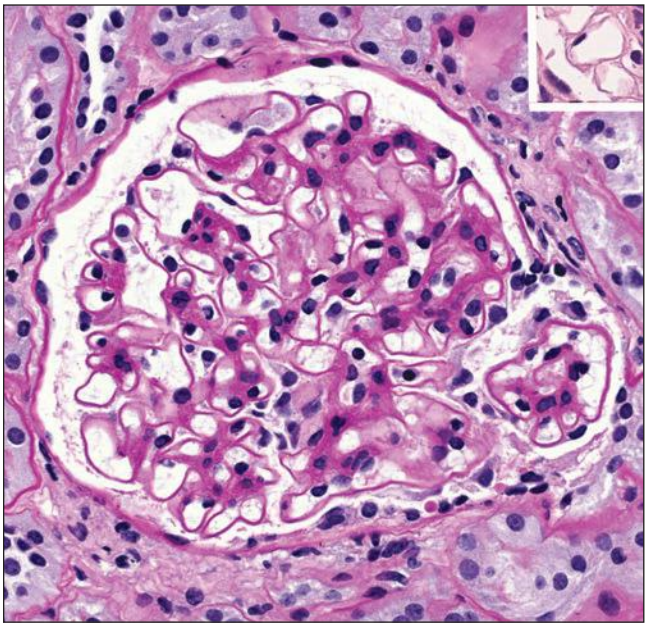


FIGURE 21.2 Glomerulus showing global uniform thickening of the capillary wall. Compare capillary wall thickness to **inset** at same magnification from patient with minimal change disease. Slight mesangial matrix increase is also present. (PAS, ×390; **inset**: H&E, ×390.)

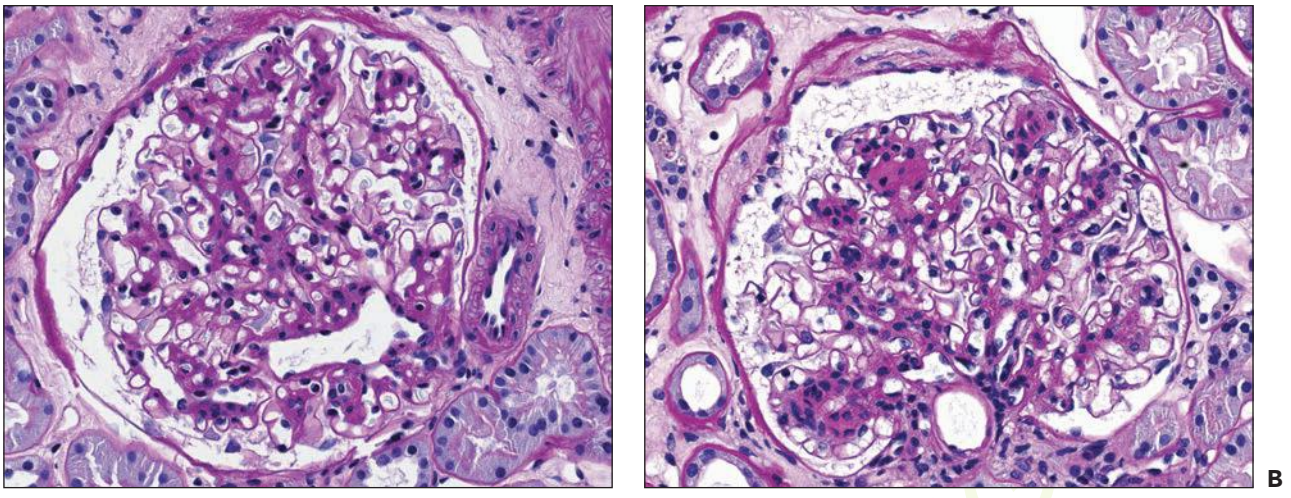


FIGURE 21.3 Glomeruli showing progression from mild mesangial matrix increase **(A)** to moderate increase in matrix with early nodule formation at 12 o'clock **(B)**. (PAS, **A:** ×260, **B:** ×260.)

Nodular Lesion The nodular lesion is the type of change first recognized by Kimmelstiel and Wilson (99). Typically, it is characterized by the accumulation of homogeneous eosinophilic material within the mesangium often appearing as a rounded accentuation of the mesangial expansion (Fig. 21.6). Formation of a Kimmelstiel-Wilson nodule is recognized when expansion of the mesangium attains a size at least one and one half times that of the normal mesangial stalk. As such, it should measure greater than 40 µm in diameter, but it may be as much as 100 µm (107). Such lesions are usually acellular, although nuclei may be arranged at the periphery. Bloodworth (107) noted at least one nodule in 25% of kidneys studied in 200 consecutive autopsies of patients with DM. Several lobules may be affected within any given glomerular tuft, and in this case, the nodules often vary in size (Fig. 21.7). On occasion, glomeruli may have a single large nodule. These variants tend to have a laminated appearance, particularly when viewed with silver or reticulin stains (Fig. 21.8); they may have a separate pathogenesis as discussed below. The number of glomeruli with nodules varies from case to case. The nodules are PAS positive, the smaller lesions staining more intensely than the larger ones. They stain green or blue with Masson trichrome, blue with Mallory stain, and black with silver stains. Falk et al. (108) reported an increase in mesangial staining for fibronectin, laminin, and types IV and V collagen in early and moderately

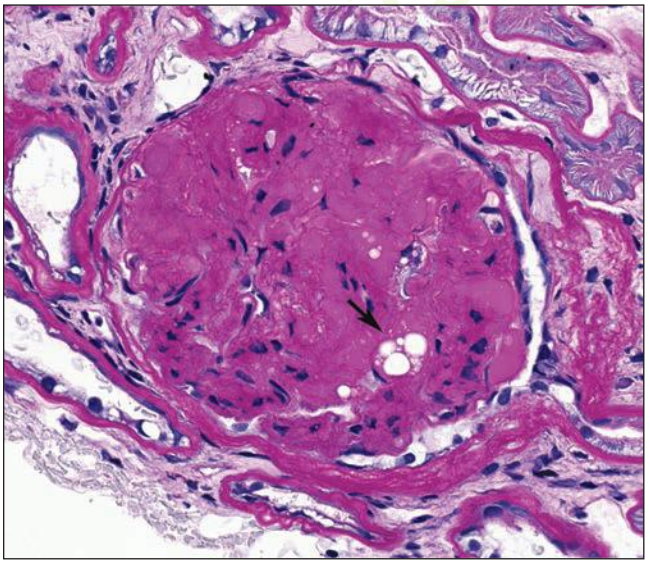


FIGURE 21.4 Solidified glomerulus filling the entire Bowman space. Note hyalinosis lesion with lipid droplets (*arrow*). (PAS, ×350.)

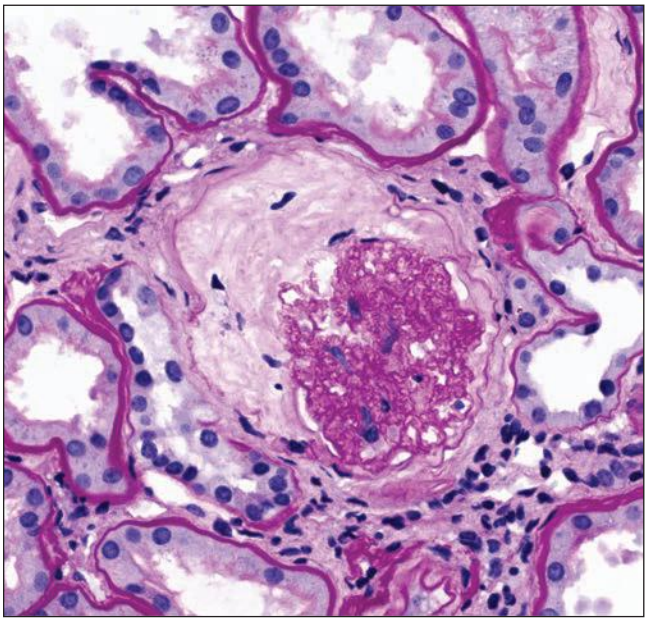


FIGURE 21.5 Ischemic change in the glomerulus, with collagen forming internal to the Bowman capsule. This change is commonly found when arterial disease is advanced. (PAS, ×380.)

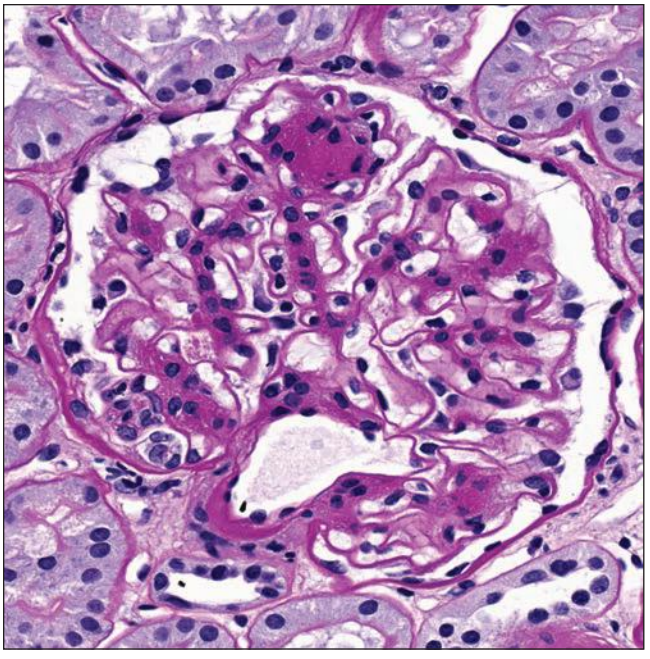


FIGURE 21.6 Glomerulus with a single well-developed Kimmelstiel-Wilson nodule at 12 o'clock. Also note uniform thickening of the GBM. (PAS, ×390.)

advanced mesangial lesions with an increase only of type V collagen in late nodules. Other investigators have demonstrated increases in collagen type 1 as well as the proteoglycans decorin and biglycan (109). More recently, aberrant laminins have been demonstrated in the matrix as the expansion progresses (110).

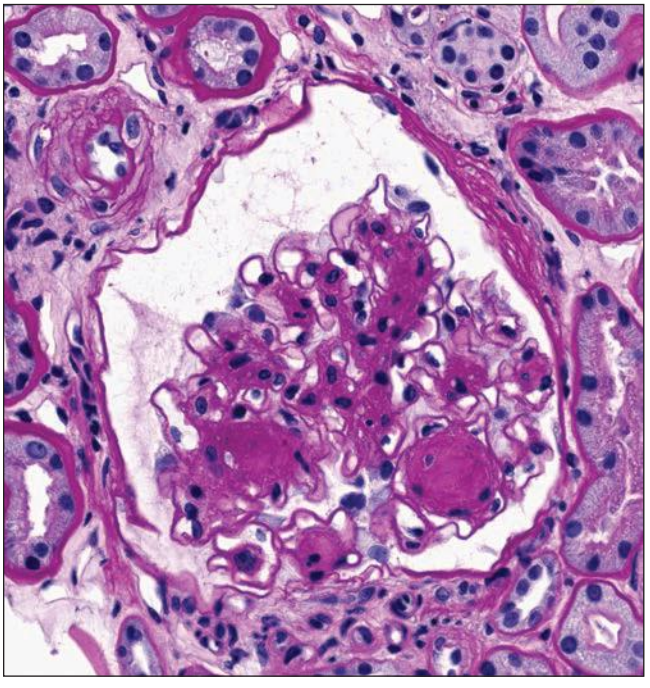


FIGURE 21.7 Glomerulus from a patient with well-developed nodular diabetic glomerulosclerosis. Several well-formed nodules with cells arranged around the periphery are present with smaller developing nodules present in other lobules. (PAS, ×350.)

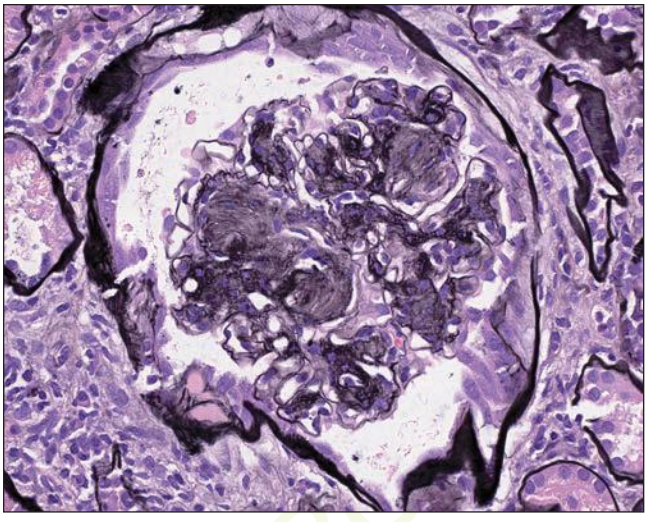


FIGURE 21.8 Glomerulus with multiple nodules with laminations. (Periodic acid-methenamine silver, ×260.)

These modifications in the composition and amount of mesangial matrix are due to both increased synthesis and decreased degradation as discussed in the "Pathogenesis" section (109).

Two populations of nodular lesions exist. The smaller, more numerous nodules arise as continued mesangial expansion of the diffuse lesion (see Fig. 21.6) (106,107). However, the larger, often solitary, and laminated nodules may originate in relation to microaneurysms (107,111–113), which are defined in this context as cystic dilations of the capillary measuring greater than 35 μm in diameter (Fig. 21.9). Investigators have observed that the frequency of microaneurysms and large nodules is similar and that microaneurysms may precede the development of large nodules (107). Such microaneurysms may arise in association with mesangiolysis (107,112–114), and a

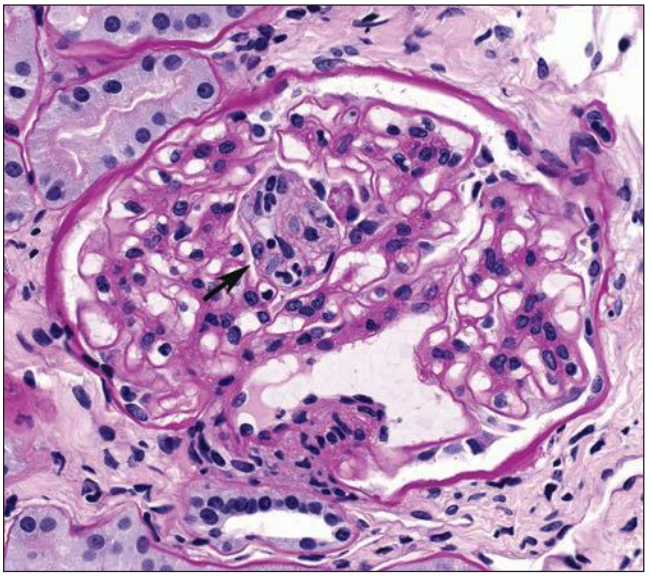


FIGURE 21.9 Glomerulus with a small microaneurysm in center containing blood elements (*arrow*) above hilar vessels. Capillary walls are thickened throughout the entire glomerulus. (PAS, ×330.)

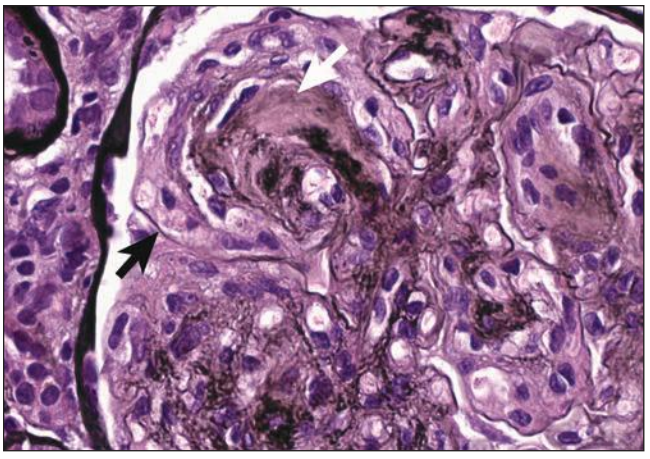


FIGURE 21.10 Glomerulus showing disintegration of the mesangial matrix (*white arrow*) and loss of anchoring points of the GBM (*black arrow*) from the mesangium. (Periodic acid-methenamine silver, ×450.)

progression of changes has been described wherein the GBM is loosened from its anchoring points as it reflects back over the mesangium (114). This change is accompanied by disintegration of the mesangial matrix (Fig. 21.10), the appearance of fibrillar material, and increasing compaction of the mesangial material resulting in the formation of several layers and a large nodule (Fig. 21.11). Agents implicated in the development of microaneurysms include platelet factors, hemodynamic factors, and possible changes in elasticity of the GBM (107,112,113). Stout et al. (115) suggested a different pathogenetic mechanism for the Kimmelstiel-Wilson nodule. Focal mesangiolysis appears first and progresses from an edematous to a proliferative

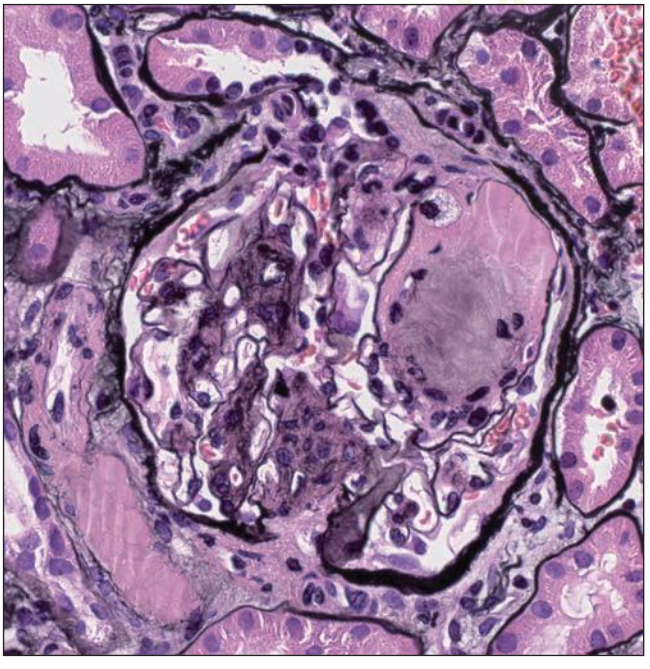


FIGURE 21.11 Portion of the glomerulus with a microaneurysm showing compaction of fibrillar material and mesangial matrix to form a large mesangial nodule. (Periodic acid-methenamine silver, ×400.)

stage characterized by a loose but organized fibrillar matrix. As this matrix condenses, the lesion changes from focal nodular mesangial expansion to a simple Kimmelstiel-Wilson nodule. Repeated injury then results in a similar progression of changes and the eventual formation of the laminated lesion called the complicated nodule. Paueksakon et al. (112) found increased plasminogen activator inhibitor-1 (PAI-1) in such lesions, particularly those in which they found fragmented red blood cells, an indicator of microvascular injury. They suggested this repeated microvascular injury caused the mesangiolysis.

Considerable overlap of the diffuse and nodular lesion occurs. However, it is useful to identify the nodular lesion because it signifies a more severe pathologic form of diabetic nephropathy (112,115) and corresponds to the presence of clinical signs and symptoms. Moreover, the presence of mesangial nodules should always arouse suspicion of diabetes. Methods to differentiate other conditions with mesangial expansion are considered in the section on "Differential Diagnosis" below.

Podocyte Loss and Hyalinosis Lesion Reduction in the number of podocytes has been documented in patients with both type 1 and type 2 DM (116,117). This reduction in podocyte number is associated with increasing proteinuria (118). Podocyte loss is associated with apoptosis, which is increased with high glucose and may also be associated with decreased synthesis of integrins involved in attachment of the podocyte to the GBM (119,120). It has been further suggested that the loss of podocytes results in denudation of the GBM and may be an initiating factor in glomerulosclerosis (121).

The hyalinosis lesion, which is another name for the so-called exudative/insudative lesion or fibrin cap, is often present in diabetic nephropathy in areas of segmental sclerosis. The initial change characteristic of this lesion is the accumulation of hyaline eosinophilic homogeneous material between endothelial cells and the GBM of the capillary loops. As the lesion evolves, the material, which represents various plasma constituents, increases in amount and eventually occludes the capillary lumen. On occasion, lipid droplets and even lipid-laden macrophages may be present within the lesion (Fig. 21.12A). The epithelial cells overlying these lesions are frequently enlarged and may show vacuoles or protein droplets. Adhesions are often observed between the glomerular lobule containing such a lesion and the nearby Bowman capsule. The material within the capillary lumen stains intensely pink with PAS (Fig. 21.12B). Use of the methenamine silver stain with PAS and hematoxylin and eosin counterstains differentiates these lesions from nodules. The combination of PAS and eosin intensifies the staining of the hyalinosis lesion producing contrast with the silver-staining nodule (Fig. 21.12C). Furthermore, the silver stains the basement membrane and reveals the luminal location of the hyalinosis lesion.

This lesion is not specific for diabetic nephropathy, although it is seen in approximately 60% of diabetic kidneys (107). It is identical to the lesion characteristic of focal segmental glomerular sclerosis and may be seen nonspecifically in certain other glomerular diseases including various forms of glomerulonephritis and reflux nephropathy. The pathogenesis of the lesion is not well understood, although its early development has been associated with endothelial injury and possible hemodynamic alterations (122). Its prevalence in diabetic kidneys increases with the severity of the nephropathy and correlates with the degree of arteriosclerosis (123). Segmental glomerulosclerosis with the hyalinosis lesion and adhesion has been associated with proteinuria (124).

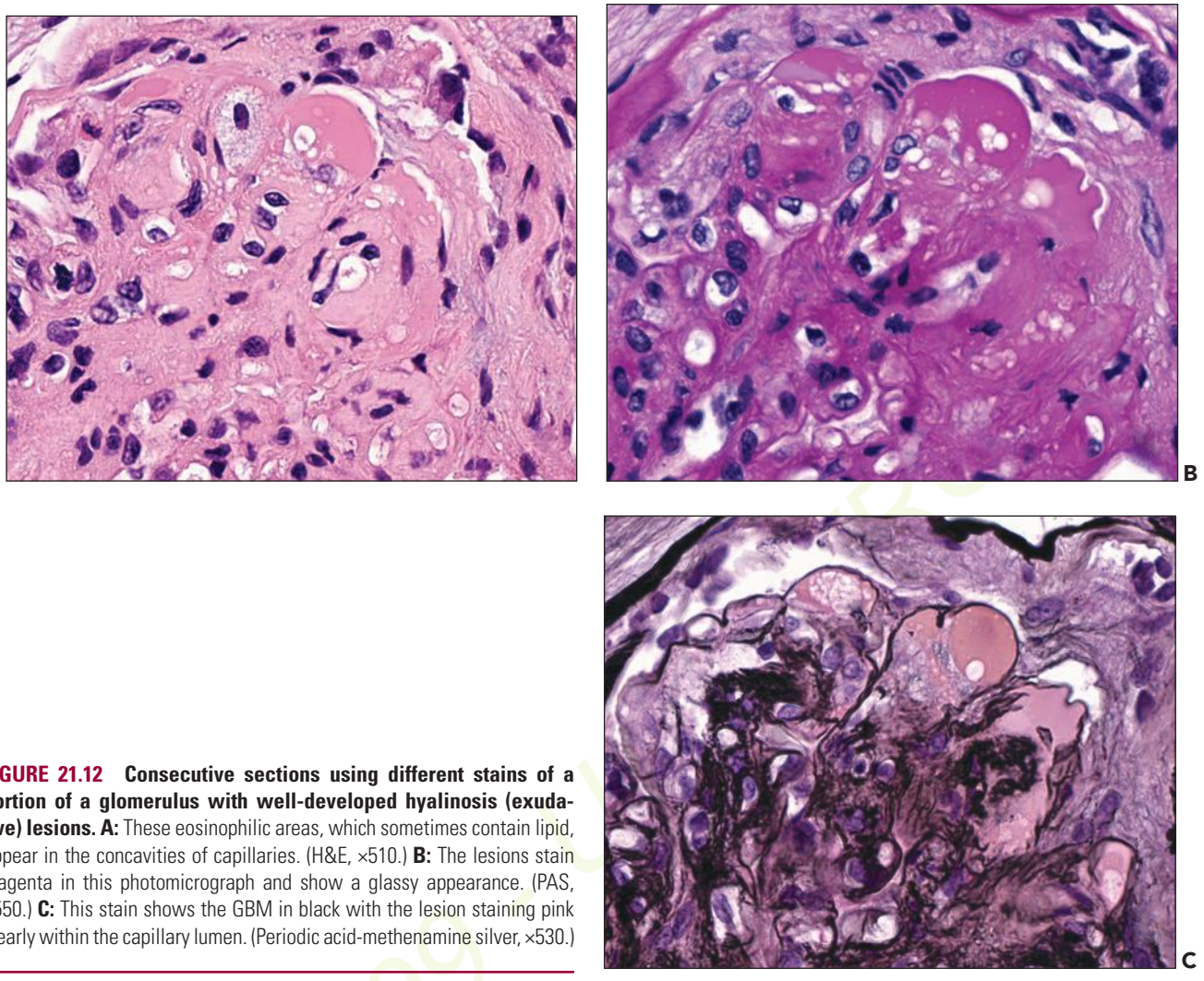


FIGURE 21.12 **Consecutive sections using different stains of a portion of a glomerulus with well-developed hyalinosis (exudative) lesions. A:** These eosinophilic areas, which sometimes contain lipid, appear in the concavities of capillaries. (H&E, ×510.) **B:** The lesions stain magenta in this photomicrograph and show a glassy appearance. (PAS, ×550.) **C:** This stain shows the GBM in black with the lesion staining pink clearly within the capillary lumen. (Periodic acid-methenamine silver, ×530.)

The lesion called the capsular drop is identified as a round, eosinophilic accumulation of material between the basement membrane and parietal epithelial cells of the Bowman capsule (Fig. 21.13). It has the same staining qualities as the hyalinosis lesion. This unusual lesion is most frequently seen in diabetes although it may be seen in other conditions (125).

Miscellaneous Glomerular Changes One of the early physiologic changes in diabetics is the occurrence of glomerular hyperfiltration accompanied by glomerular hypertrophy. Some of the patients with hyperfiltration then proceed to develop the characteristic histologic changes of diabetic nephropathy described earlier, namely, increased mesangial matrix, the nodular lesion, increased thickness of the GBM, and the hyalinosis lesion. As these lesions evolve, the solidified glomeruli fail to undergo contraction, and the result is a population of large obsolescent glomeruli (see Fig. 21.4). A second population of smaller obsolescent glomeruli may also emerge in response to the vascular disease that is a frequent companion to the glomerular disease in diabetes. Thus, these smaller glomeruli are identical to those seen in ischemic renal disease in other conditions (see Fig. 21.5). Furthermore, they are present in a

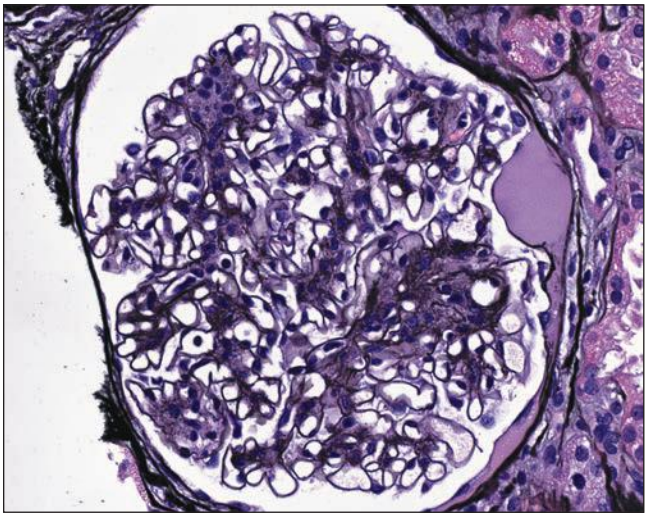


FIGURE 21.13 Glomerulus with capsular drop at 3 o'clock. (Periodic acid-methenamine silver, ×260.)

distribution expected for vascular disease; that is, they occur in stripes perpendicular to the capsular surface within the distribution of the affected vessel (126,127).

Atubular glomeruli have also been seen in diabetic nephropathy (124,128). Atubular glomeruli are defined as those glomeruli that have open glomerular capillaries but have lost their connection to the proximal tubule and presumably do not produce filtrate (129). Accurate determination of these glomeruli requires serial sections. However, small glomeruli surrounded by tissue with marked tubular loss are likely to be atubular (Fig. 21.14). Najafian et al. (128) using the appropriate morphometric techniques found that 17% of glomeruli in diabetics were atubular and that an additional 51% were attached to atrophic tubules. They found that these glomeruli could account for much of the variation in GFR in diabetic patients. Furthermore, glomeruli with such glomerulotubular abnormalities were found in 77% of glomeruli in patients with proteinuria compared to only 4% of glomeruli in patients with microalbuminuria (124).

Tubules

The tubules generally show changes that reflect the degree of glomerular alterations. Obsolescent glomeruli (of either variety) show atrophy of adjacent tubules with decreased size of epithelial cells and diminished luminal diameters. Similar changes are also seen in the tubules that belonged to now atubular glomeruli. Apoptosis has been detected in both proximal and distal tubules of the diabetic kidney (130) and represents a possible mechanism for the loss of the tubular cells. The tubular basement membrane is often thicker than expected for the degree of atrophy (Fig. 21.15). Occasionally, proximal tubular epithelial cells are finely vacuolated and contain lipid; this usually occurs in patients manifesting the nephrotic syndrome.

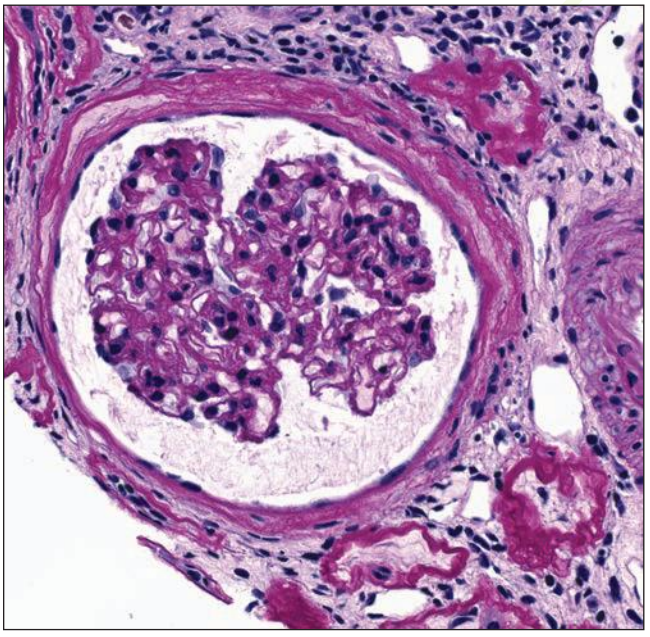


FIGURE 21.14 Glomerulus that has likely lost its connection to its proximal tubule, although serial sections would be needed to confirm this change. Note marked atrophy of surrounding tubules and wrinkling of glomerular capillary loops. (PAS, ×320.)

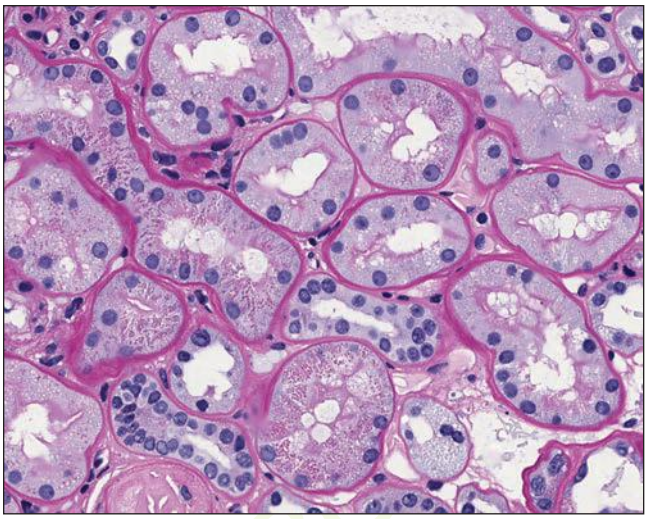


FIGURE 21.15 Light micrograph showing mild thickening of the tubular basement membrane in tubules with slight tubular injury. (PAS, ×320.)

The straight portion (S3) of the proximal tubules may show the glycogen-containing Armanni-Ebstein change (Fig. 21.16), but this is seen only rarely.

Interstitium

Interstitial fibrosis is common in the diabetic kidney and may be accompanied by chronic inflammatory infiltrates composed chiefly of T lymphocytes and macrophages (131–133) (Fig. 21.17A). A Th1 process has been implicated in type 1 DM (131). Eosinophils are also frequently noted within the interstitial infiltrate (Fig. 21.17B). In some cases, this represents a hypersensitivity-type reaction superimposed on the diabetic nephropathy, but this is not necessarily the case. Mast cells are increased in kidneys of patients with type 2 DM and renal disease (134). The number of mast cells was correlated

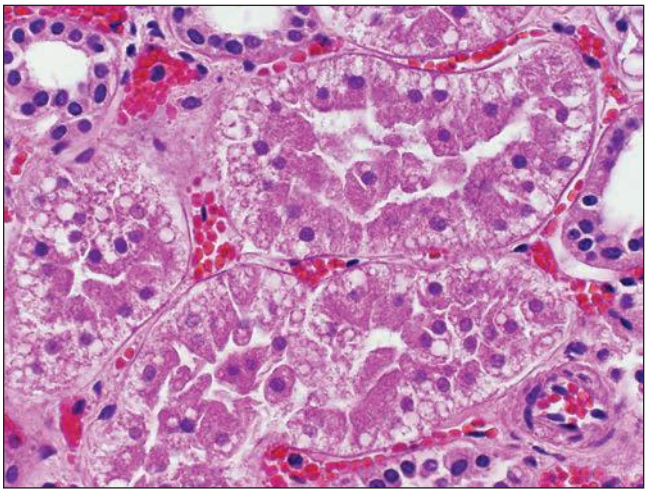


FIGURE 21.16 Light micrograph showing tubules with Armanni-Ebstein lesion characterized by a subnuclear vacuole representing glycogen. (H&E, ×380.) (Courtesy of Dr. Melvin Schwartz.)

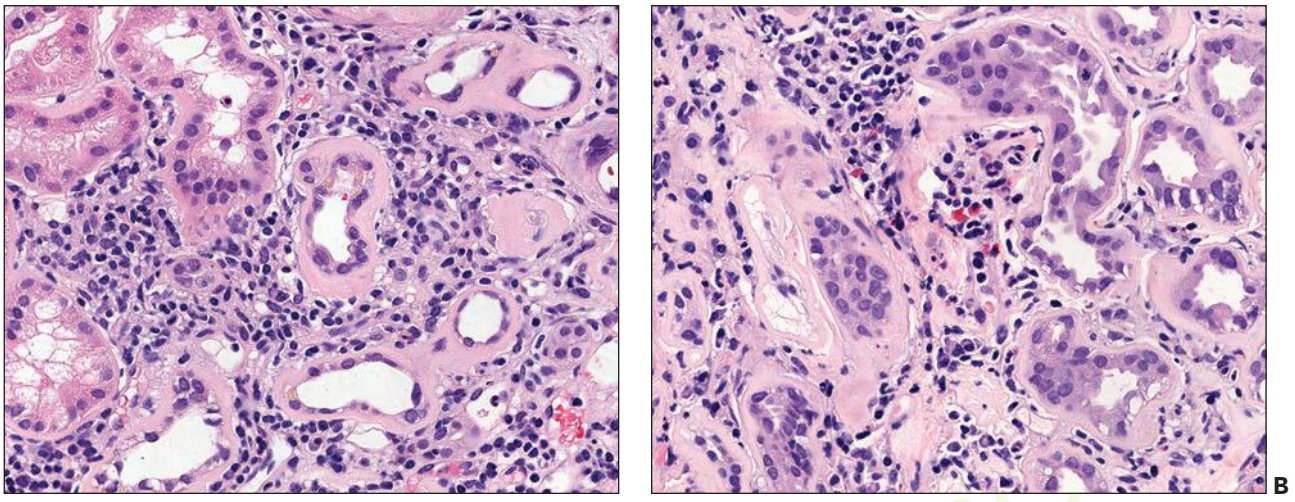


FIGURE 21.17 Light micrographs of typical patterns of interstitial inflammation in diabetic nephropathy. A: Dense inflammation is composed chiefly of lymphocytes and macrophages with rare plasma cells. Note the variable thickening of the tubular basement membrane surrounding damaged tubules. (H&E, ×320.) **B:** Cluster of eosinophils is found within an area of interstitial inflammation and damaged tubules. More plasma cells are also seen within this focus. (H&E, ×320.)

to the increase in interstitial volume as well as to decrease in estimated GFR (eGFR) (134). It is now considered that these inflammatory cells play an active role in the pathogenesis of the interstitial fibrosis. The increase in interstitial volume is due largely to increase in cells at early stages of diabetic nephropathy associated with only mild glomerular changes (135). Increases in collagen and other matrix components occur later in the disease (135). The presence of interstitial fibrosis, particularly when accompanied by inflammatory infiltrate, correlates inversely with renal survival (133,136). Lane et al. (137) demonstrated that mesangial expansion, arteriolar hyalinosis, global glomerular sclerosis, and interstitial expansion are interrelated. However, the progression in each compartment is not stereotypic for all patients. Thus, in some patients, the severity of interstitial disease may be greater than that of glomerular lesions, whereas in others, the reverse may be true.

Blood Vessels

Both arteries and arterioles invariably show the typical changes of arteriosclerosis and arteriolosclerosis, respectively. In arteries, this type of injury is manifested by varying degrees of intimal thickening accompanied by reduplication of elastic lamina. Hyaline arteriolosclerosis is a frequent and early manifestation of diabetic renal disease and is more pronounced in diabetes than in other diseases of the kidney. It is characterized by often striking hyaline deposition in arterioles (Fig. 21.18), and both afferent and efferent limbs may be affected. Østerby et al. (138) noted increase in vascular disease associated with more severe glomerular damage. These investigators assessed the ratio of matrix to media in arterioles from diabetic patients with and without microalbuminuria (138,139) and found that this ratio was increased in patients with microalbuminuria. This finding suggested that arteriolar matrix accumulation may be similar to increased matrix elsewhere in diabetes and can occur early in the course of the disease. Furthermore, this change progresses with the duration of disease (138). Three-dimensional analysis of the vascular pole of the glomerulus demonstrated new vessel formation with anastomoses between glomerular capillaries near the hilus and peritubular capillaries (140). The presence of intrarenal microaneurysms in arterioles has been documented using microangiography (111).

Immunofluorescence Microscopy

Immunofluorescence techniques have been used in several studies. The typical finding is the occurrence of linear staining along the glomerular capillary walls with immunoglobulin G (IgG) (Fig. 21.19) (141–144). The intensity of the staining

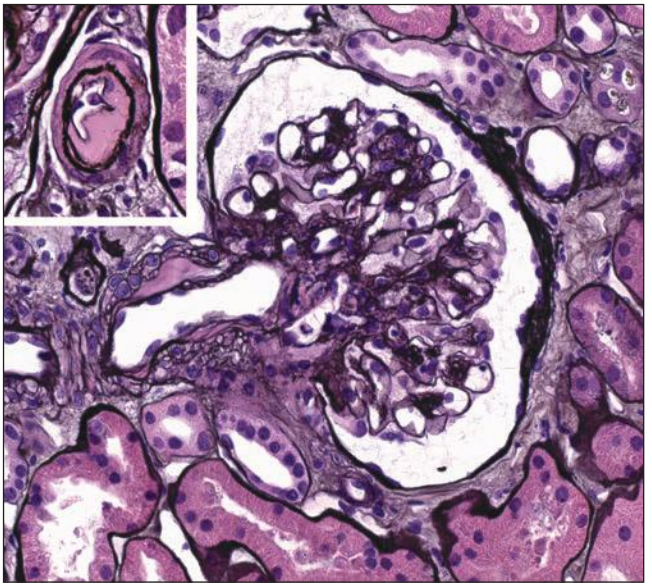


FIGURE 21.18 Hyaline arteriolosclerosis affecting the afferent arteriole in a patient with diabetes. The glomerulus shows diffuse mild mesangial expansion. The **inset** demonstrates a cross-section of a similar lesion. (Periodic acid-methenamine silver, ×300; **inset**: ×430.)

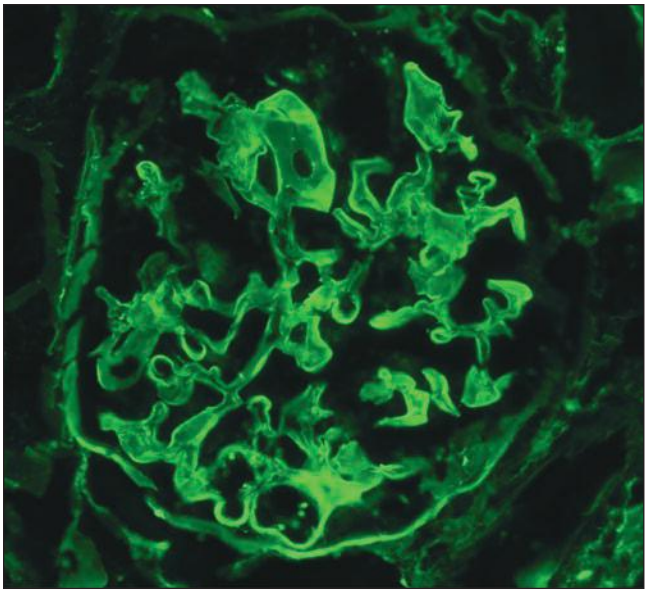


FIGURE 21.19 Immunofluorescence micrograph of a glomerulus with a diabetic nodule at 6 o'clock using antiserum to IgG. One sees positive linear staining along capillary walls. (×320.)

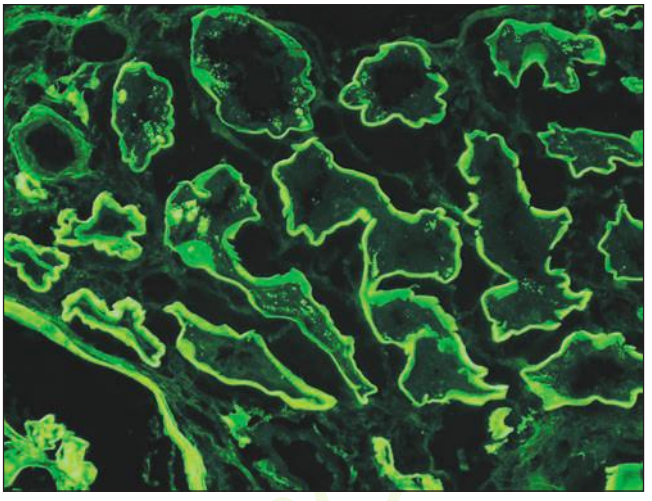


FIGURE 21.20 Immunofluorescence micrograph showing linear staining for albumin along the tubular basement membrane. (×400.)

varies among individual patients and does not correspond to the severity of the glomerular lesions (144). Linear staining of capillary walls has also been reported with IgM, the third component of complement (C3), fibrinogen, and albumin (141,144). Staining of the mesangium and Kimmelstiel-Wilson nodules is rarer and usually shows fainter staining than that reported for capillary walls (141,144).

Hyalinosis lesions usually stain brightly with IgM and C3, as they do in the identical lesions found in focal segmental glomerular sclerosis, pyelonephritis, and elsewhere. IgM and C3 are also present in arteriolar hyalin in vessels showing hypertensive changes. Hyalinosis lesions may also contain fibrinogen and lipoprotein (142) or fibrinogen, complement, β-lipoprotein, and small amounts of IgG (143).

Linear staining for IgG and albumin has also been reported along the tubular basement membranes and Bowman capsule in patients with advanced diabetic glomerulosclerosis (145) (Fig. 21.20). The staining was thought to reflect structural changes in the renal extracellular membranes, which permit entrapment of serum proteins, possibly as a result of changes in permeability. It is now believed that advanced glycosylation end products may bind to the basement membranes and change their properties (146).

Electron Microscopy

In the capillary wall, morphologic changes are present in all three layers, GBM, visceral epithelium, and the endothelium. These will be discussed in turn. Most authors agree that increase in the thickness of the GBM is the earliest change (105,147,148) (Fig. 21.21). Huang (149), using guanidine treatment, showed that the increase in basement membrane thickness was due to increased amounts of material from the epithelial rather than from the endothelial cells. The isolated finding of the thickened GBM is a possible manifestation of prediabetes as demonstrated in one study in 23 patients who presented with proteinuria greater than 0.5 g/d sometimes accompanied by hematuria but without clinical evidence of diabetes (150). Two years later, seven patients remained normoglycemic, six had fasting blood glucose between 110 and 125 mg/dL, three had impaired glucose tolerance, and seven had become diabetic (150). As duration of diabetes increases, greater variability in thickness of the GBM is observed (151,152), but interindividual variation and interglomerular variation contribute to the large standard deviations (105). Vogler et al. (152) studied 15 patients with type 1 diabetes and found areas of attenuation of the GBM in six patients. In part, this change is related to the thinner GBM in microaneurysms (Fig. 21.22). Østerby and Nyberg (151) observed similar basement membrane thinning in severely affected diabetic kidneys. In such cases, the GBM measured as little as 100 nm and comprised 1% to 5% of the total capillary length in individual glomerulus. Changes in the biochemical composition of the GBM have been reported by various investigators. Spiro (153) first recorded an increase in hydroxylation of lysine and in the number of disaccharide units. Additional work from his laboratory has shown decreased heparan sulfate and laminin (154). Other authors, using immunohistochemical localization techniques, detected a change in charge density resulting from alteration in the location of the heparan sulfate rather than an absolute decrease in the amount (155,156). Kim et al. (157) showed a change in the chains of collagen type IV in the GBM.

Changes in the foot processes of visceral epithelial cells are variable. At times, they remain discrete, whereas other cases have differing degrees of effacement (101,105,107,148,151,152). Østerby et al. (151) measured a mean width of the foot process of 352 nm in diabetics as compared with a mean width of 224 nm in nondiabetic control patients. Widening of foot processes appears with the onset of microalbuminuria (158). Pagtalunan et al. (117) in a study of Pima Indians did not document widened foot processes until the stage of clinical nephropathy. Furthermore, these investigators were the first to note absolute loss in the number of visceral epithelial cells during clinical

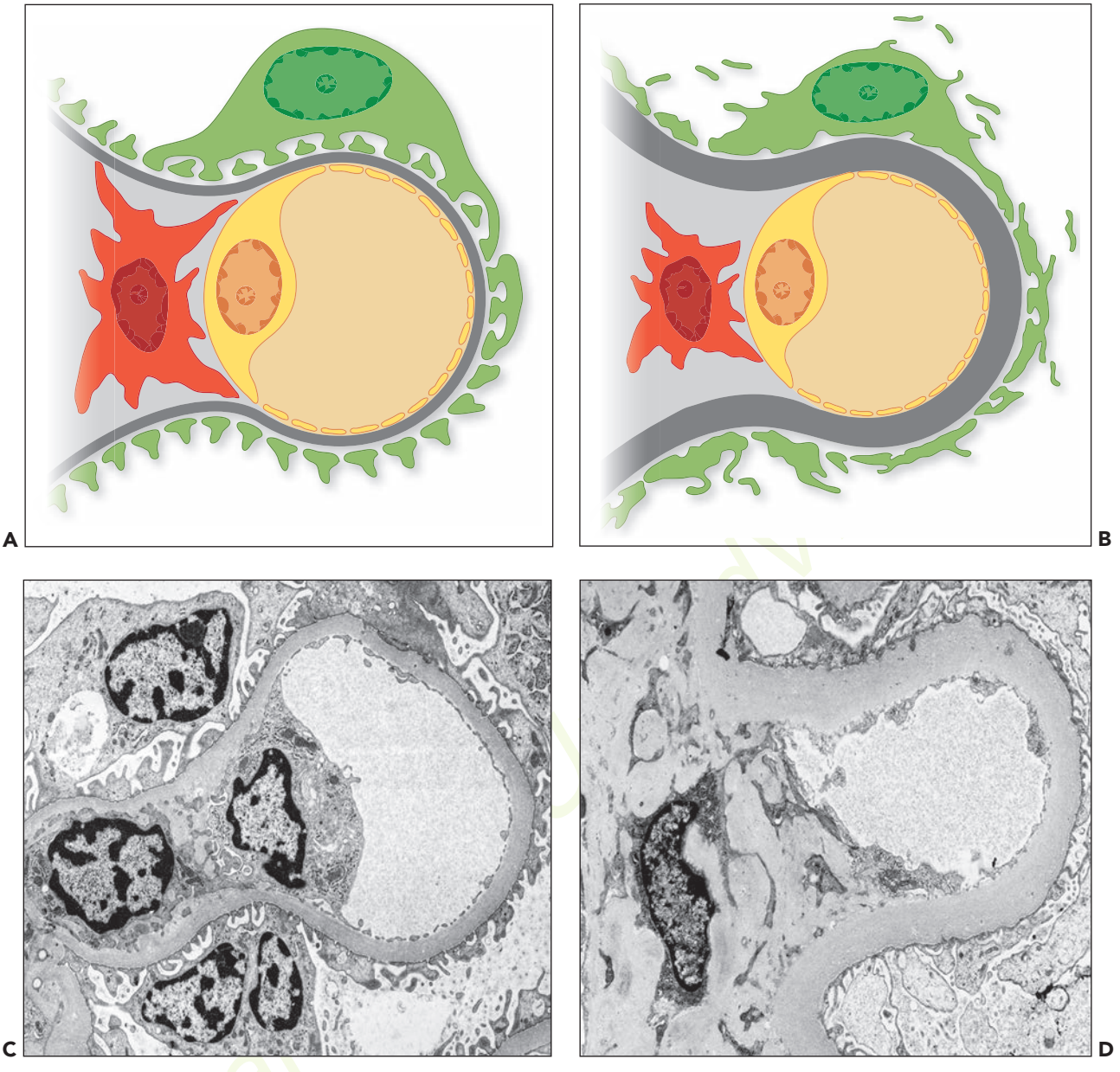


FIGURE 21.21 **A:** Drawing of normal capillary. **B:** Drawing of capillary with diabetic changes. **C:** EM of normal capillary. **D:** EM of capillary with diabetic changes.

nephropathy concurrent with mesangial expansion. Podocyte depletion has been confirmed in patients with either type 1 or type 2 DM by other investigators (116,118,159,160). Toyoda et al. (161) studied foot process detachment in patients with type 1 DM and found that 22% of GBM was not covered by intact foot processes in diabetic patients with proteinuria as compared to 11% in such patients with microalbuminuria and 4% of diabetics with normoalbuminuria. Weil et al. (159,160) using a more conservative technique found a smaller percentage of foot process detachment in type 2 diabetic patients, but the differences were significant between patients with proteinuria (1.48% detachment) and those with normoalbuminuria (0.41% detachment) or microalbuminuria (0.37% detachment). Such foot process detachment has been associated with glomerular sclerosis in a number of different glomerular lesions and may be important in the progression of diabetic nephropathy as well (161). Furthermore, the visceral epithelial cells play an important role in maintaining endothelial cells so that loss of podocytes may influence these cells as discussed below.

Endothelial cell changes have been largely overlooked in diabetic nephropathy until recently although endothelial dysfunction has been documented for years. Reduction in endothelial fenestration was first documented in the rat with alloxan-induced diabetes in 1980 (162). Similar studies were not undertaken in human patients with diabetes for more than

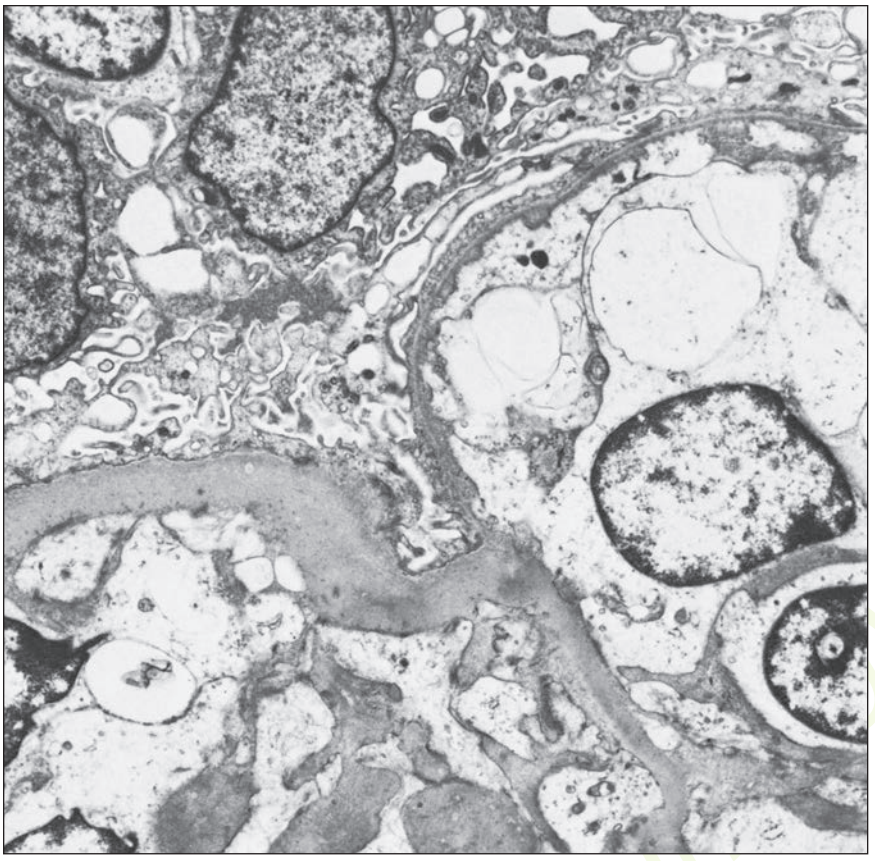


FIGURE 21.22 Electron micrograph of a portion of a glomerulus showing marked attenuation of the basement membrane in a loop to the right with an abrupt transition as it reflects over the mesangium. This thinning of the GBM is associated with the formation of microaneurysms. (Uranyl acetate and lead citrate, ×9700.)

25 years. Toyoda et al. (161) in a study of patients with type 1 DM found that the fractional surface of fenestrated endothelium was reduced from 41% in controls to 25% in patients with proteinuria. Patients with normoalbuminuria or microalbuminuria showed 32% fractional surface of fenestrated endothelium. Weil et al. (159) found similar results in patients with type 2 DM. We now recognize that vascular endothelial growth factor (VEGF) secreted by the podocyte has an important role in control of endothelial structure (163). However, Weil's study did not find any difference in the degree of fenestration near areas of foot process detachment (159). Satchell suggests several possible explanations, including the finding that actual detachment of foot processes was quite rare (164). Furthermore, he suggests that other factors such as the endothelial glycocalyx may play a role in glomerular permeability in this setting (164,165).

The hyalinosis lesion occurs within capillary loops and appears on electron microscopy as an accumulation of homogeneous electron-dense material between the endothelial cell and the GBM. As more material collects, it fills the capillary lumen (Fig. 21.23). In obsolescent glomeruli, remains of the hyalinosis lesions formed earlier persist as denser areas within increased matrix and the GBM. Similar material may also be present within the mesangium (107,166). One must distinguish such hyalinosis accumulations from immune deposits, although this distinction may be difficult. This material is identical to that seen in the subintima of arterioles with hyaline arteriolosclerosis. Furthermore, the capsular drop is made up of similar material lying between the Bowman capsule and the parietal epithelium.

Mesangial widening and nodules are due to increased synthesis of mesangial matrix (149) and decreased degradation secondary to cross-linking of glycosylated collagens (167,168) (Fig. 21.24). Additional factors are considered in the section

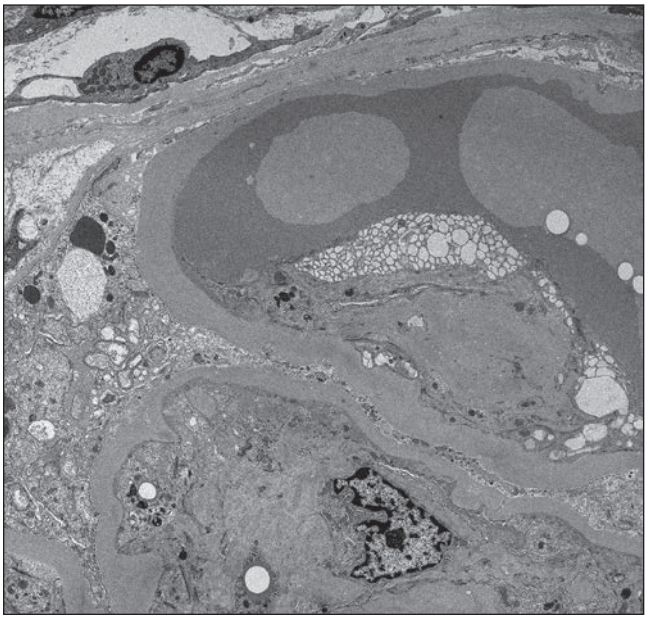


FIGURE 21.23 Electron micrograph of a portion of a glomerulus showing a hyalinosis lesion in a capillary loop and marked foot process effacement. (Uranyl acetate and lead citrate, ×4700.)

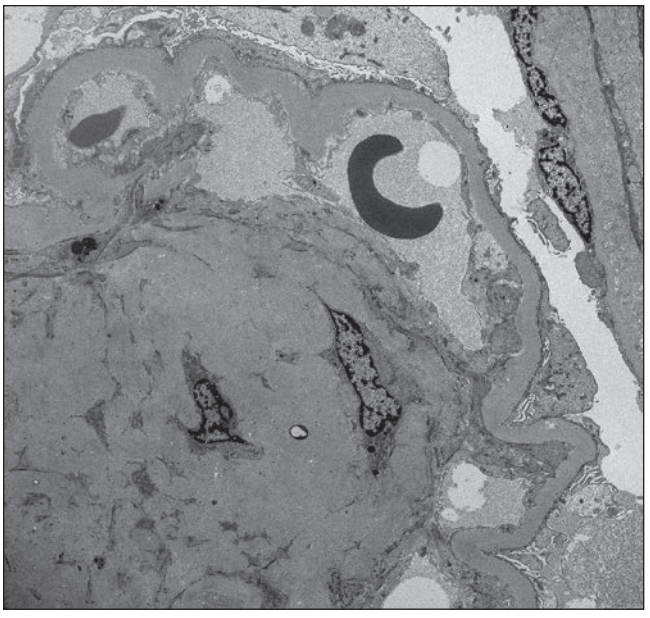


FIGURE 21.24 Electron micrograph of a Kimmelstiel-Wilson nodule showing increased matrix and relationship to capillary loops with thickened GBM and effacement of foot processes. (Uranyl acetate and lead citrate, ×3000.)

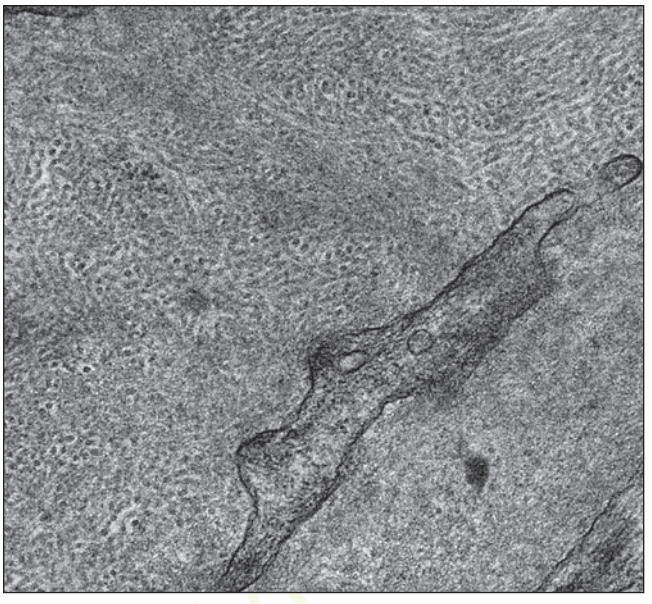


FIGURE 21.25 Electron micrograph showing typical appearance of diabetic fibrillosis characterized by densely packed collection of microfibrils. (Uranyl acetate and lead citrate, ×47,100.)

on pathogenesis. Dachs et al. (169) noted that the first change consisted of widening of the usually delicate strands of mesangial matrix with increase of the numbers of mesangial cells. The number of mesangial cells and cellular processes may be slightly increased within the expanded mesangial matrix. These observations have been confirmed by morphometric analysis. Steffes et al. (170) showed increase in volume fraction of mesangial matrix per glomerulus and in mesangial cells per glomerulus in patients with type 1 diabetes compared with controls. The volume fraction of mesangium increases over the duration of the diabetes (147). Cell debris manifested as small calcific deposits, remnants of cell membranes, and scattered organelles are often present (166). On occasion, dense collections of fibrils measuring between 10 and 25 nm are present in the mesangium (171,172) (Fig. 21.25). The presence of such diabetic fibrillosis does not affect the course or prognosis (171,173). The fibrils can be distinguished from amyloid by their lack of staining by Congo red (172). Mesangiolysis has been described in association with the formation of microaneurysms (111,114). Some nodules develop loosening of the matrix, resulting in detachment of endothelial cells and loss of the anchoring points of the GBM to the mesangium (166). These alterations are thought to precede the exaggerated mesangial expansion of the single large nodules with laminated texture that are associated with microaneurysms (113).

The major component of the increased matrix is type IV collagen (155,156,174). Other components that are increased include type V and VI collagen, laminin, and fibronectin (175). Suzuki et al. (175) examined glomerular and interstitial expression of the mRNAs of metalloproteinase-3, tissue inhibitor of metalloproteinase-1, and type IV collagen in diabetic nephropathy. Expression of integrins is increased in all cell types in diabetic patients with moderate increase in the mesangial matrix (176). As the mesangial matrix increases to severe degrees, the expression of integrins increases in parallel in mesangial cells, whereas it remains the same in epithelial cells with decreased expression in endothelial cells.

Diabetic Nephropathy in Type 2 Diabetes Mellitus

The early studies in the morphologic changes in diabetic nephropathy were performed in patients with type 1 or in mixed populations. As the prevalence of type 2 diabetes has increased, more studies have been performed utilizing renal biopsies from patients with type 2 DM allowing the determination of any differences from the kidneys of patients with type 1 DM. In general, these studies showed many similarities with regard to the basic lesions of diabetic nephropathy, that is, diabetic nephropathy is characterized by increased GBM thickness, diffuse mesangial sclerosis with nodule formation, hyalinosis, microaneurysms, and hyaline arteriolosclerosis in both types of diabetes (177–185). However, several authors have suggested that the renal disease of patients with type 2 diabetes is more heterogeneous due to additional comorbidities such as cardiovascular disease, hypertension, aging, and other diseases (179–182,184,186). These investigators agree that many of these patients have typical diabetic lesions usually associated with other complications such as retinopathy. However, a subpopulation show only nondiabetic glomerular disease or alterations associated with vascular disease such as more severe interstitial fibrosis, glomerulosclerosis without prominent mesangial expansion, and arterio- and arteriolosclerosis (180,181,184).

Most patients with type 2 DM and renal disease show changes consistent with diabetic nephropathy with or without concurrent vascular disease (182,184,187). Schwartz et al. (184) examined 34 renal biopsies from patients with type 2 DM with proteinuria, renal insufficiency, and hypertension. Two had nondiabetic renal disease (one with IgA nephropathy,

the other membranous glomerulonephritis). Seventeen patients had typical nodular disease with Kimmelstiel-Wilson nodules, more marked GBM thickening, and more pronounced hyaline arteriolosclerosis. The remaining 15 patients had diffuse mesangial sclerosis consistent with diabetic nephropathy but did not have nodules. Mazzucco et al. (182) examined 393 renal biopsies from patients with type 2 DM in a multicenter study. They defined four classes of histologic findings. Class 1 was defined as typical diabetic nephropathy and was present in 40% of the biopsies. Within this group, 13% had predominantly nodular change, 34% had no nodules, and 53% showed a mixed nodular and diffuse pattern of mesangial expansion. Class 2 was characterized by ischemic changes with collapsed sclerotic glomeruli, tubular atrophy, and arterio- and arteriolosclerosis and was seen in 15% of the biopsies. Diabetic changes were not present in these cases. Class 3 was defined by the presence of another glomerular disease and was observed in 45% of the biopsies. Within this group, the glomerular disease was superimposed on diabetic changes in 38% of cases (class 3a). The remaining 62% did not have typical diabetic renal disease (class 3b).

The standard criteria used to determine the need for renal biopsy in patients with DM and evidence for renal disease include normal funduscopic findings, hematuria (without urologic etiology), rapid onset of acute renal insufficiency, and/or nephrotic syndrome within a short duration of the diabetes or in the presence of normal hemoglobin A1c. The study by Mazzucco et al. (182) cited above also examined the effects of using different criteria to determine need for biopsy. Approximately half of the biopsies were performed only if a disease other than diabetes was suspected. The other half was performed for proteinuria greater than 0.5 g/d with or without hematuria and/or altered renal function. The data from the group using more restricted criteria showed 29% with diabetic nephropathy only, 15% with vascular changes, and 56% for those with nonglomerular disease with or without diabetic changes. The results in the group using an unrestricted policy were 52%, 16%, and 32%, respectively, demonstrating a reversal of the diabetic versus nondiabetic disease with similar degree of vascular disease (182). In a recent Chinese study, more strict criteria were used for renal biopsy in type 2 diabetics resulting in 93.5% of patients with nondiabetic renal disease (186). A total of 18.5% of the patients had diabetic changes with or without other alterations.

Two groups of investigators have examined the role of the renal biopsy in predicting outcome in type 2 DM. Ruggenenti et al. (188) used the same three classes as defined above and devised a renal tissue injury score based on damage to all four compartments. They found that a combination of proteinuria and this injury index predicted progression to ESRD. Patients with proteinuria ≤2 g/24 hours or greater than 2 g/24 hours and a renal injury index less than seven never progressed to ESRD. However, all patients with proteinuria greater than 2 g/24 hours and an injury index of greater than 13 progressed to ESRD over a median of 1.6 years. Mazzucco et al. (189) using a similar renal injury index found comparable results.

Classification

A classification scheme has been developed recently, which may be applied to diabetic nephropathy in both types 1 and 2 DM (190). This consensus classification uses only glomerular lesions in order to make it easy to use and reproducible. Four classes are defined, one of which is subdivided. Class I shows mild or nonspecific changes with GBM thickening proven by electron microscopy. GBM thickening is defined as greater than 395 nm in females and greater than 430 nm in males older than 9 years of age when directly measuring the GBM (191). The definition of thickened GBM is 471 nm for women and 521 nm for men using the orthogonal intercept method (192). Class II shows mesangial expansion defined as greater than the width of two mesangial nuclei in two lobules in greater than 25% of glomeruli. This class is further subdivided into mild (less than the diameter of a capillary lumen) and severe (greater than the diameter of a capillary lumen). Class III is defined by the presence of at least one Kimmelstiel-Wilson nodule and less than 50% globally sclerotic glomeruli. Class IV demonstrates global sclerosis in greater than 50% of glomeruli in combination with lesions from the other three classes.

This proposed classification requires validation and evaluation to determine its usefulness. Two studies have applied it to biopsies from patients with type 2 DM (193,194). Oh et al. (193) examined 126 renal biopsies from patients with type 2 DM and proteinuria greater than 1 g/d, renal involvement without retinopathy, renal involvement within 5 years of diagnosis, or unexplained hematuria or azotemia. They split the biopsies into three groups with pure diabetic nephropathy (n = 50), nondiabetic renal disease (n = 65), and mixed (n = 11) and further analyzed the pure diabetic nephropathy group using the classification described above (190). They also used semiquantitative assessment of glomerular size, mesangial proliferation, mesangial matrix, interstitial fibrosis, arteriolar hyalinosis, and arteriosclerosis. At the end of a 5-year follow-up period, ESRD was present in no patients with class I or IIA changes, in 41.7% with class IIB, in 44.4% with class III, and 61.7% with class IV (193). The classification did correlate to urine protein to creatinine ratio and eGFR at the time of biopsy; however, this was due to the strong correlation to class IV to these factors as no differences were present with classes II and III. In fact, class IIb and class III showed similar clinical and pathologic features. Okada et al. (194) studied 69 patients with pure diabetic nephropathy and a mean follow-up of 59 months (range 6 to 180 months). They used the classification described above (190) for glomerular lesions and also assessed interstitial fibrosis and tubular atrophy (IFTA), interstitial inflammation, arteriolar hyalinosis, and arteriosclerosis (194). Using multivariate analysis, these authors found that IFTA were the only significant predictor of renal survival and not classification by glomerular lesions (194). Furthermore, no difference was found in the renal prognosis in a comparison between class IIA and IIB with class III.

Fioretto and Mauer (195) suggest that this classification is not yet ready for clinical use but that it does represent a first step in developing a useful scheme. They point out that its use in type 2 diabetes may not be possible for all cases in view of the variety of glomerular lesions seen in the biopsies from this population. This idea is supported by the fact that the papers cited above used the classification in type 2 diabetics but only evaluated biopsies with pure diabetic nephropathy, avoiding the issue of mixed lesions (193,194). Fioretto and Mauer suggest that addition of an evaluation of arteriolar hyalinosis might be useful as a means of distinguishing global glomerulosclerosis due to diabetes from that due to other forms of renal diseases first described by Harris et al. (196). They are also concerned about the use of mild increases in GBM thickness as a

defining feature of diabetic nephropathy and the use of a single Kimmelstiel-Wilson nodule to indicate a more severe lesion as such nodules may be present in the absence of clinical evidence of renal disease (195).

Etiology and Pathogenesis

Hyperglycemia plays a central role in the pathogenesis of diabetic nephropathy by activating similar intracellular signaling events in all four compartments of the kidney. The initial manifestations of diabetic renal involvement can be ascribed to glomerular capillary dysfunction with hyperfiltration and microalbuminuria followed by development of further structural and functional abnormalities affecting the glomerular, tubulointerstitial, and vascular compartments. The cellular events induced by high glucose ambience include increased flux of polyols and hexoseamines, generation of AGEs and ROS, and activation of the PKC, transforming growth factor–β-Smad-mitogen-activated protein kinase (TGF-β-Smad-MAPK), and Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathways and of the G protein signaling pathway. These abnormalities along with aberrant expression of cyclin kinases, ECM proteins, metalloproteinases, and their inhibitors result in altered cell cycle and growth and subsequent accumulation of ECM proteins, a characteristic morphologic feature of diabetic nephropathy in humans. In addition to the biochemical events, mechanical injury mediated by increased glomerular intracapillary pressure due to impaired autoregulation of the local intrarenal renin-angiotensin-aldosterone system can further potentiate the injury. The following section integrates the foregoing physiologic and pathologic features of diabetic nephropathy with these factors to explain our current understanding of the pathogenesis more fully.

Hemodynamic Changes and Hyperfiltration

The prevalence of hyperfiltration in type 1 diabetes varies from less than 25% to more than 75%, while the corresponding figures for type 2 diabetes are significantly lower, ranging between 0% and more than 40% (197). Several factors, both biologic and methodologic, may contribute to the wide variation in hyperfiltration prevalence. That the GFR is increased early in the course of DM was first shown by Stalder and Schmidt in 1959 (198) and later confirmed by several studies. In 1971, Mogensen (199) showed a 40% increase in the GFR in 11 patients with new-onset diabetes when compared with age-matched controls. Insulin therapy tended to return these elevations in the GFR to normal. The four factors regulating GFR are renal plasma flow, oncotic pressure, transcapillary hydraulic pressure, and the glomerular ultrafiltration coefficient (K_f). There is compelling evidence indicating that hyperfiltration in diabetic patients is mediated by increases in glomerular plasma flow, transcapillary hydraulic pressure, and the glomerular K_f, which is the product of filtration surface area and glomerular capillary hydraulic permeability (200). A correlation exists between the degree of increase in the GFR and the filtration surface area of the glomeruli in diabetic patients (201). Increased glomerular intracapillary pressure may potentially accelerate the renovascular complications of diabetes via cross-talk between hemodynamic and metabolic factors in the diabetic environment (202).

Evidence that hyperfiltration is an important risk factor for subsequent increase in UAE and later development of diabetic nephropathy in patients with type 1 diabetes has been shown in several studies (203–206). Yip et al. (205) compared 25 patients with type 1 diabetes and hyperfiltration with a second group with a normal GFR for 10 years. The two groups had similar initial levels of blood pressure, UAE, and glycosylated hemoglobin. UAE at the end of the study was higher in the group with hyperfiltration, and it was an independent predictor of blood pressure at the end of the study. Rudberg et al. (206), in a study of adolescents with type 1 diabetes, showed similar results in which metabolic control of blood glucose was the most important factor in initiation of renal injury, followed by clinical duration of diabetes. In addition, systemic blood pressure was more important in progression than in the initiation of diabetic nephropathy. A meta-analysis of 10 type 1 diabetes studies concluded that the presence of hyperfiltration at baseline more than doubled the risk of developing micro- or macroalbuminuria at follow-up (207). However, two additional single-center studies addressing the relationship of hyperperfusion and albuminuria have arrived at different conclusions. The first study of 2318 adults with type 1 diabetes (208) showed no significant differences between the eGFR in patients with type 1 diabetes and normoalbuminuria from those expected in the general population. Furthermore, type 1 diabetic patients with a higher eGFR were also no more likely to develop microalbuminuria over a median of 5.2 years of follow-up than those with normal eGFR. Similarly, a second study of 426 normoalbuminuric patients with a follow-up of 5, 10, and 15 years reported that the risk of progression to microalbuminuria was not increased by hyperfiltration (209).

As mentioned earlier, the prevalence of glomerular hyperfiltration in patients with type 2 diabetes also varies widely (210–217), and its prognostic significance is not well defined (212,215,217,218). In studies of the natural history of diabetic nephropathy among the Pima Indians with type 2 diabetes, GFRs at baseline among those with normal glucose tolerance, impaired glucose tolerance, newly diagnosed diabetes, and diabetes of more than 5 years' duration with microalbuminuria or macroalbuminuria were 123, 135, 143, 155, and 124 mL/min, respectively (215). During 4 years of follow-up, the GFR increased by 18% in the patients who initially had newly diagnosed diabetes; the rate declined by 3% in those with microalbuminuria at baseline and by 35% in those with macroalbuminuria. The authors concluded that GFR is elevated at the onset of type 2 diabetes, remains elevated while normal albumin excretion or microalbuminuria persists, and declines after the development of macroalbuminuria. Vedel et al. (212) studied the GFR in both normoalbuminuric (n = 39) and microalbuminuric (n = 158) patients with type 2 diabetes. The GFR in patients without microalbuminuria did not differ from that in controls. However, hyperfiltration defined as greater than 2 standard deviations above the mean GFR was identified in 37% of patients with microalbuminuria. Multiple regression analysis demonstrated correlations between the GFR and glycosylated hemoglobin, urinary sodium excretion, age, and known duration of diabetes in these patients. The authors of this study concluded that in patients with type 2 diabetes, glomerular hyperfiltration is a putative risk factor for progression. A recent study also showed that elevated GFR in patients with type 2 diabetes predicts its subsequent decline, which may occur without worsening of albuminuria (216). In contrast, another recent study by Magri and Fava (217) found

no association between hyperfiltration and albuminuria in patients with type 2 diabetes during the early stages of diabetic nephropathy. This study also showed that the decline of GFR and albuminuria followed a parallel course during the later stages of the disease raising the possibility that they share common pathophysiologic mechanisms. The beneficial effects of good metabolic control to reduce GFR toward normal levels within days to weeks have also been shown in several studies in both type 1 and type 2 diabetes (199,219). Some data seem to indicate strict control of blood glucose levels inhibits the progression of albuminuria; however, it does not offer any difference in clinical kidney disease outcome (220–222).

Although several studies support the notion of glomerular hyperfiltration being a potentially significant contributor to the pathogenesis and progression of diabetic nephropathy, some studies clearly indicate that hyperfiltration alone is not sufficient for the evolution of diabetic nephropathy. Schmitz et al. (223) examined kidney function in 29 patients with a single kidney. The three patients with type 1 diabetes had had a single kidney for 18 to 30 years. Two of the three patients had elevation in both the GFR and kidney size, but none had UAE greater than 20 mg/d. All three had normal blood pressure and serum creatinine levels. Thus, these three patients showed no ill effect from prolonged hyperfiltration, a finding suggesting that other factors must be present to produce glomerulosclerosis. Hyperfiltration is also influenced by protein intake, and restricting dietary protein has been shown to reduce glomerular hyperfiltration. Zeller et al. (224) found that dietary protein and phosphorus restriction resulted in a slowing of progression of renal disease independent of blood pressure and glycemic control that was comparable in the two experimental groups. However, whether amelioration of hyperfiltration in patients with early diabetic nephropathy is renoprotective is still uncertain (218).

The molecular mechanisms leading to hyperfiltration are complex, and many factors seem to have influence on its development. The concept of local intrarenal hypertension (i.e., intraglomerular hypertension) as a potential cause for hyperfiltration arose from micropuncture studies in diabetic rats (202). These rats disclosed intraglomerular hypertension despite having normal systemic blood pressure suggestive of impaired autoregulation due to differential dilation of the afferent and efferent arterioles (200). Such impaired autoregulation might be triggered by activation of the RAS with local production of angiotensin II (225). In support of this notion is the ability of mesangial cells as well as podocytes not only to synthesize angiotensin II but to express its receptors, which sets the stage for the regional activation of RAS (226,227). This may explain the success of inhibition of ACE in the treatment of diabetic nephropathy. Increased production of angiotensin II due to dysregulation of the regional RAS may also account for the hyperplasia and hypertrophy during the early and late stages of diabetic nephropathy via the up-regulation of various growth factors and cytokines such as TGF-β, connective tissue growth factor (CTGF), interleukin-6, monocyte chemoattractant protein-1 (MCP-1), and vascular endothelial growth factor-A (VEGF-A) (200,225). Hyperglycemia and increased production of ROS in the diabetic milieu could instigate regional RAS activation by up-regulating angiotensinogen and renin production in the mesangial cells (228–230). Sustained-stretch stress on the glomerular capillaries as well as mesangial cells due to increased intracapillary pressure caused by efferent arteriolar vasospasm may activate various signaling pathways with subsequent rise in blood pressure, mesangial cellular proliferation, ECM production, and decreased expression of the ECM-degrading enzymes (231–234). Mechanical stretch responses in the mesangial cells include induced expression of TGF-β and its receptor, activation of PKC and p38/MAPK, induction of MCP-1 with up-regulation of intercellular adhesion molecule-1, and the generation of ROS (235,236). Glomerular capillary stretch stress also induces VEGF-A expression by the podocytes (121,237), leading to down-regulation of α3β1 integrin and nephrin with detachment from the underlying basement membrane and ensuing proteinuria (121,238,239).

There is also evidence indicating that insulin-like growth factor (IGF-I) plays an important role in the early renal growth in diabetes (240–243) and that hyperfiltration may, at least in part, be mediated by IGF-I (244). Growth hormone and glucagon, both with well-known influence on GFR, are increased in diabetic patients (245,246). Reduction of serum glucose levels is accompanied by diminished levels of glucagon and growth hormone with continuous infusion of insulin by pump. These reductions are correlated with decrease in the GFR (247). The GFR is also affected by parathyroid hormone, vasopressin, eicosanoids, and angiotensin II (248,249). Esmatjes et al. (250) compared the GFR and urinary prostaglandin excretion in 21 patients with recent-onset type 1 diabetes and 15 controls. These investigators confirmed the findings of earlier studies of increased GFR and renal plasma flow. When they administered lysine acetylsalicylate, a prostaglandin synthetase inhibitor, they found a reduction in both the GFR and renal plasma flow that did not occur in the control group. These investigators suggested that this finding supported the idea that the increase in the GFR often seen in diabetic patients is due at least in part to mediation by prostaglandins. A specific role of cyclooxygenase (COX)-2 in glomerular hyperfiltration has also been demonstrated. A study by Cherney et al. (251) evaluated the effects of COX-2 inhibition on renal function in normotensive and normoalbuminuric patients with uncomplicated type 1 diabetes. In patients with hyperfiltration, COX-2 inhibition partially blunted the hyperfiltration, while patients with normal GFR exhibited an increase in GFR under these conditions. These findings were interpreted as an indication that hyperfiltration and normofiltration are distinct physiologic states. Another study by the same group of investigators showed that the dependence on vasodilator prostaglandins in type 1 diabetes is greater in women than in men (252). This finding is consistent with recent evidence that sex hormones may contribute to the pathogenesis of diabetic nephropathy (253). Other substances that have been implicated in increasing GFR in diabetes include kinins (254), atrial natriuretic peptide, hyperglycemia, and other factors in the abnormal metabolic environment (211). Nugent et al. (255) showed a loss of autoregulation in response to endothelin in diabetic patients. This alteration could make these patients more susceptible to hyperfiltration in response to alterations in glomerular hemodynamics.

Glomerular hypertrophy and hyperfiltration have also been observed in STZ-induced diabetes in experimental animals along with glomerular hyperperfusion, increased glomerular capillary hydrostatic pressure, unchanged systemic oncotic pressure, and unchanged or slightly elevated ultrafiltration coefficient (K_f) (256–258). Rats made diabetic with STZ

and partially treated with insulin to maintain moderate levels of hyperglycemia develop increased whole-kidney and single-nephron GFR (256). The increment in single-nephron GFR is due to increases in transcapillary hydraulic pressure difference and initial glomerular plasma flow rate (256). When glomerular pressure is lowered by the use of ACE inhibitors, glomerular injury is decreased in this model (259,260), a finding suggesting a role for increased glomerular pressure in this form of injury. ACE inhibitors also reduce the morphologic alterations seen in this model (261). Treatment with insulin normalized glomerular pressure and renal hypertrophy (262,263). However, Bank et al. (264) showed that increased glomerular pressure alone is not sufficient to cause glomerular lesions in diabetic rats. Some of the glomerular hemodynamic changes have been attributed to the effects of diabetes on afferent arteriolar resistance resulting in vasodilation. However, the precise mechanisms for the changes in glomerular hemodynamics remain undefined. Reports on nitric oxide (NO) as a potential mediator of vasodilation and hyperfiltration in diabetes are conflicting (265–267). Additional suggested mechanisms include increase in glomerular synthesis of prostaglandins (268,269), alterations in the intrarenal renin-angiotensin axis (270), changes in the polyol pathway (271), diminished bioavailability of proinsulin connecting peptide (C-peptide) (272), open K_{ATP} channels affecting preglomerular microvascular smooth muscle function (273), altered tubuloglomerular feedback (274,275), and increased production of IGF-1 (258). Hypertrophy and hyperfiltration are reduced in STZ-induced diabetes via modulation of IGF-1 bioactivity by inhibiting nitric oxide synthase supporting the role of IGF-1 mediating hyperfiltration (258). Some data also suggest that these events might be further modulated by proinflammatory mediators and cytokines (276).

In summary, hyperfiltration occurs early in the course of diabetes, and some data indicate correlation with the later appearance of diabetic nephropathy. The mechanism responsible for the increased filtration is not well defined, although several humoral factors, including hormones, growth factors, and prostaglandins, have been implicated. Increased glomerular pressure, an element in the mediation of hyperfiltration, has been demonstrated to affect UAE in diabetics. However, other factors are probably also necessary for the development of diabetic glomerulosclerosis in any particular patient.

Metabolic Factors

The relationship between metabolic abnormalities and the development of renal disease is complex. Hyperglycemia-induced complications are mediated by several metabolic pathways, among which accumulation of AGEs with abnormalities of the glycosylation of macromolecules and increased glucose flux through the polyol pathway seem to be the most important. The altered glucose metabolism will ultimately result in renal functional and structural abnormalities via changes in gene expression and augmented oxidative stress leading to increased ECM production and cellular senescence (Fig. 21.26). These are discussed in turn. The subject has been reviewed by Kanwar et al. (277).

The Role of Altered Metabolic Pathways

In diabetes, the increased amount of glucose taken up by the cells is channeled into various metabolic pathways, among which the hexosamine pathway, polyol pathway, and the myo-inositol pathway are the best characterized. Under normal circumstances, glucose undergoes phosphorylation through conversion to glucose-6-phosphate (G6-P) followed by isomerization to fructose-6-phosphate (F6-P). Under high glucose ambiance, F6-P is diverted into the hexosamine pathway with

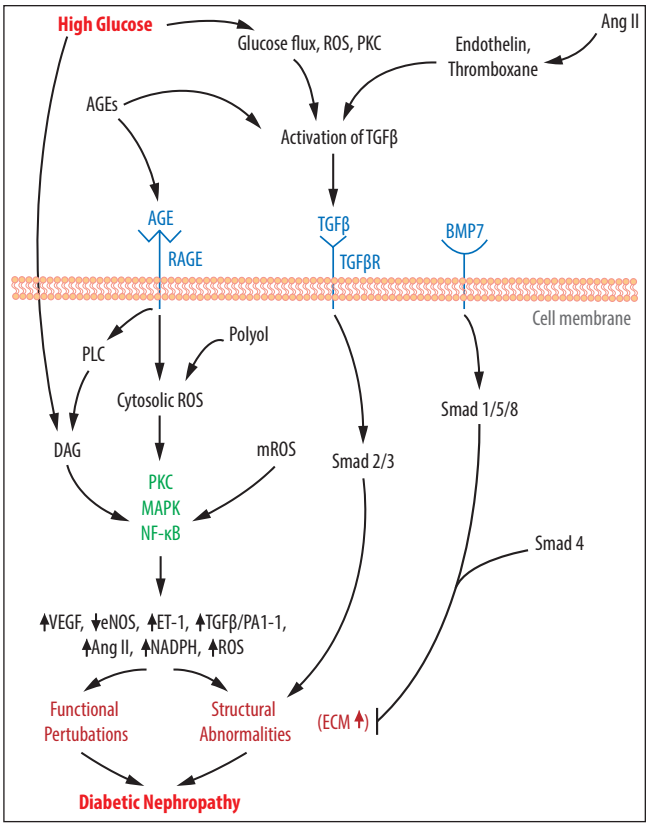


FIGURE 21.26 Summary of cellular signaling events due to altered glucose metabolism in the pathogenesis of diabetic nephropathy. As a shift from the normal glycolysis, excess glucose is channeled into the polyol and hexosamine pathways, leading to generation of DAG, augmentation of oxidative stress via increased production of reactive oxygen species (ROS), and activation of protein kinase C (PKC). Downstream events follow, which ultimately result in increased extracellular matrix (ECM) deposition and cellular senescence. Advanced glycation end products (AGEs), generated in excess in the hyperglycemic milieu, alter the structure and function of a number of intra- and extracellular molecules, modulate cell activation, signal transduction, and the expression of cytokines and growth factors through receptor-dependent and receptor-independent pathways. AGE-RAGE (receptor for AGE) interactions followed by the generation of activated phospholipase C (PLC) and DAG along with cytosolic and mitochondrial ROS (mROS) increase the expression and activity of PKC, mitogen-activated protein kinase (MAPK), and NF-κB, leading to altered expression of a number of genes such as vascular endothelial growth factor (VEGF), endothelial nitric oxide synthase (eNOS), transforming growth factor–β (TGF-β), PAI-1, and further generation of ROS. TGF-β plays a central role in promoting ECM deposition via interaction with Smad2/3. TGF-β, generated by activation of its latent form by excess glucose, AGEs, ROS, and angiotensin II (Ang II), up-regulates the transcription of various ECM genes, connective tissue growth factor, and tissue inhibitor of metalloproteinase and down-regulates MMPs with a net result of increased matrix deposition. BMP-7 has an opposing effect to the actions of TGF-β via activation of Smad1/5/8 that binds to Smad4 followed by translocation to the nucleus and inhibiting DNA binding of some of the transcription factors.

the formation of UDP-*N*-acetylglucosamine, a precursor of ECM proteins such as the proteoglycans (278). A rate-limiting enzyme in this process also modulates promoter activities of ECM proteins such as TGF-β1 and PAI-1 (278). Excess glucose is also channeled into the polyol pathway resulting in changes in the polyol-inositol metabolism important in the pathogenesis of some diabetic complications (279). AR reduces aldehydes generated by ROS to inactive alcohols, and glucose to sorbitol, using NADPH as a cofactor. Sorbitol is oxidized by sorbitol dehydrogenase (SDH) to fructose using NAD^+ as a cofactor. In cells where AR activity is high enough to deplete reduced glutathione (GSH), oxidative stress is augmented. At normal glucose concentrations, metabolism of glucose by this pathway is limited because AR exerts relatively low affinity for glucose and the precise physiologic role of the pathway is unclear. However, as much as 30% of the glucose could be channeled through this pathway in diabetes (279). In hyperglycemia, increased intracellular glucose activates AR, which results in increased conversion of glucose to sorbitol and decreased intracellular NADPH content. This, without concomitant increase in SDH, leads to accumulation of sorbitol (280). Although there are several mechanisms that have been proposed to explain the role of polyol pathway in the diabetic renal complications, the precise pathogenetic mechanism(s) remains unclear. In addition to induction of oxidative stress, osmotic-induced vascular damage has also been postulated. Finally, glucose is also channeled into the myoinositol pathway. Glucose competes with the high-affinity transporters of myoinositol into cells, thus depleting tissue levels of this substrate during periods of hyperglycemia (281). The decrease in myoinositol leads to a reduction in certain sodium-potassium adenosine triphosphatases through a decline in phosphoinositide metabolism. There may also be an effect on PKC activity and adenosine release (281). Although the exact effect on the development of renal disease is unknown, investigators have hypothesized that changes in sodium-potassium adenosine triphosphatase may lead to alterations in the mesangial cells and smooth muscle cells of arterioles with alteration of vascular tone or modification of the responses to neurotransmitters and hormones (282).

In patients with type 2 diabetes, increased expression of AR has been detected in the glomerular mesangial areas, and decreased tubular expression of SDH has been associated with interstitial fibrosis and thickened tubular basement membranes (283). Heesom et al. (284) demonstrated that patients with type 1 diabetes and nephropathy had decreased frequency of the $Z + 2$ allele of the AR gene compared to a group of patients with type 1 diabetes who did not have nephropathy after 20 years. Pedersen et al. (285) administered ponalrestat, an AR inhibitor, to 10 normoalbuminuric patients with type 1 diabetes for 6 months and compared them with 10 other patients receiving a placebo. The experimental group experienced a significant reduction in the GFR. In microalbuminuric type 2 diabetic patients, 5-year treatment with epalrestat, another AR inhibitor, prevented the progression of incipient diabetic nephropathy (286).

Increased expression of AR has been demonstrated in the kidneys of experimental animals with diabetes (283,287,288). Transgenic mice overexpressing human AR developed pathologic changes in the kidney with vascular thrombi and fibrinous deposits in the Bowman capsule (289). However, no pathologic changes in other organs were observed, and the renal changes were not typical for diabetic nephropathy. While most published studies point toward activation of the polyol pathway and sorbitol accumulation in diabetes, there are also some conflicting results. Bank et al. (271) found that administration of an AR inhibitor to STZ-treated diabetic rats reduced single-nephron GFR and plasma flow with no effect on serum glucose. Other experimental evidence has shown that treatment with AR inhibitors reduces the tissue depletion of myoinositol and the occurrence of diabetic complications in rats treated with STZ (290). Goldfarb et al. (291) used two different maneuvers to explore the possible mechanisms of the polyol pathway in the development of diabetic nephropathy. Inhibition of the polyol pathway caused by administration of sorbinil, an AR inhibitor, prevented glomerular hyperfiltration. They also used dietary supplementation with myoinositol, which also resulted in reduction of glomerular hyperfiltration. McCaleb et al. (292) showed reduction of UAE with the use of an AR inhibitor. Unfortunately, other long-term studies administering AR inhibitors to rats have not shown a consistent reduction in renal injury (293,294). Other studies compared the effects of inhibition of AGEs and inhibitors of the polyol pathway and showed that inhibitors of AGEs made a greater contribution to the amelioration of the renal lesions (295). In type 1 diabetic rats, short-term oral administration of epalrestat prevented the increase in UAE and the reduction of anionic sites on the lamina rara externa of the glomerular capillary basement membrane (296). In a 5-year study in dogs, AR inhibition failed to prevent thickening of the basement membranes in the kidney (297).

Formation of Advanced Glycation End Products and Resultant Renal Injury

The presence of glycosylated proteins in diabetic patients was first recognized by Rahbar in 1968 (298). The relationship between control of blood glucose levels and amount of glycosylated hemoglobin was discerned by Koenig et al. (299) in 1976. Since that time, investigators have shown that many intracellular, extracellular, and plasma proteins, including hemoglobin, albumin, lipoprotein, myelin, tubulin, lens protein, and various matrix proteins such as collagen may become glycosylated with varying physiologic effects (300).

AGEs are formed via the nonenzymatic Maillard reaction between reducing sugars and amino residues. This is a relatively slow process that, under physiologic conditions, occurs predominantly in long-lived proteins such as collagen and lens proteins. In diabetes, hyperglycemia and oxidative stress favor the reversible glycosylation of proteins, lipids, and nucleic acids with the attachment of glucose to the amino groups forming Schiff bases at a rate proportional to the serum glucose concentration. Additional chemical rearrangement forms the more stable but still a reversible early glycosylation product known as the Amadori product. Slow complex rearrangement then proceeds to the formation of irreversible AGEs, such as carboxymethyl lysine (CML), pentosidine, imidazolone, and pyrraline. This alteration is permanent, and the AGEs continue to accumulate (301). Also, formation of intracellular AGEs may occur from reactive carbonyl intermediates triggered by oxidative stress at a much faster rate than formation from glucose (302).

AGEs alter the structure and function of other intra- and extracellular molecules and modulate cell activation, signal transduction, and the expression of cytokines and growth factors through receptor-dependent and receptor-independent

pathways (303–306). Several properties of AGEs contribute to their pathologic effects including resistance to proteolytic digestion, high reactivity causing cross-link formation and trapping of proteins, lipid oxidation, and inactivation of nitric oxide (168). The resistance to proteolytic degradation by matrix metalloproteinases (MMPs) and increased formation of cross-links contribute to mesangial matrix accumulation, a prominent pathologic alteration in diabetic nephropathy. Cross-linking of matrix proteins may also increase permeability of basement membranes perhaps via reducing their electronegativity (270,307). Intracellular AGEs can modulate various signaling events such as the activation of PKC, MAPK, and transcription factor nuclear factor κB (NF-κB) (308). Ensuing changes in the expression of growth factors and cytokines such as TGF-β will contribute to the pathologic deposition of ECM proteins.

In addition to altering the structure and function of proteins, AGEs may also exert their effects through interactions with a number of specific cell surface receptors or binding proteins, such as RAGEs (receptor for AGEs) (304), AGE-R1, AGE-R2, AGE-R3 (galectin-3), SR-A (scavenger receptor A), CD36, and LOX-1 (oxidized low-density lipoprotein receptor-1) (309), among which RAGE is the best characterized. A low-level constitutive expression of RAGE has been documented in the podocytes in normal human kidneys, but not in the mesangium or glomerular endothelium (310). In diabetic glomeruli, up-regulation of RAGE has been demonstrated in the podocytes (310). Upon binding to their receptors on various cell types such as macrophages and mesangial and endothelial cells, AGEs can induce expression of cytokines and growth factors, among which there are several key mediators of diabetic complications including VEGF, CTGF, TGF-β1, IGF-I, platelet-derived growth factor (PDGF), tumor necrosis factor–α (TNF-α), and interleukins (309). Expression of growth factors may promote synthesis of matrix proteins by mesangial cells (311). In cell cultures, binding of AGEs to receptors on bovine aortic endothelial cells results in procoagulatory changes with up-regulation of endothelial tissue factor and down-regulation of endothelial thrombomodulin (TM) expression (312). AGE-RAGE interaction in cultured human umbilical vein endothelial cells induces expression of vascular cell adhesion molecule-1 (VCAM-1) (313), which can prime the diabetic vasculature for monocyte adhesion and increased permeability. AGEs may also induce production of endothelin, which, in addition to the quenching effect of AGEs on nitric oxide, accentuates vasoconstriction (314). AGE-induced generation of ROS along with quenching of NO is likely to modulate PKC and MAPK activities and to activate NF-κB and activator protein-1 (AP-1), further promoting ECM protein production and deposition (277,315).

Both in vitro and in vivo data indicate that TGF-β1 plays a particularly important role in mediating the effects of the AGE-RAGE interaction on cell growth and matrix homeostasis (316,317). In vitro data show that AGEs cause transcriptional up-regulation of TGF-β1 via PKC or oxidative stress. In mesangial and glomerular endothelial cell cultures, glycated albumin and AGE-rich proteins increase TGF-β1 levels, PKC activity, and ECM expression (318). In mesangial cell cultures, the AGE-RAGE interaction has been shown to activate the TGF-β1-Smad signaling pathways with subsequent mesangial cell hypertrophy and fibronectin synthesis mediated by autocrine Ang II generation via induction of intracellular ROS (317). The authors of this study postulated that AGE-RAGE–mediated ROS generation participates in the development of glomerulosclerosis by activating the Ang II-TGF-β1 signaling pathways with subsequent effects in inducing increased ECM expression.

Another growth factor, CTGF, acts as a downstream mediator of TGF-β1–induced fibrosis (319). Up-regulation of glomerular expression of CTGF has been shown in rats following repeated intravenous injections of AGEs (320). The up-regulation took place predominantly through a TGF-β1–independent pathway. In mesangial cell cultures exposed to AGEs, the significant increase in collagen IV and fibronectin production could be completely prevented by pretreatment with anti-CTGF antibody (320). These results suggest that AGE-induced CTGF expression plays an important role in the development of diabetic nephropathy.

The immunohistochemical expression of AGEs has been shown in human kidneys. Nishino et al. (321) demonstrated the presence of AGEs in various locations in kidney biopsies of diabetic patients. The AGEs were detected in the intimal thickening of vessels (92% of diabetics and 43% of controls), in hyaline arteriolosclerosis (46% of diabetics and 5% of controls), and in glomeruli (21% of diabetics and none of the controls). Tanji et al. (310) demonstrated the expression of two subclasses of AGEs, that is, CML and pentosidine in renal biopsies of diabetic patients. Pentosidine represents intermolecular cross-links between modified proteins, while the function of CML is less well defined. The expression of CML and pentosidine was similar in diffuse and nodular diabetic nephropathy. CML was the major AGE detected in the mesangium, GBMs, tubular basement membranes, and vessel walls. Pentosidine was preferentially located in interstitial collagen and was less consistently observed in vessel walls, mesangium, GBM, and tubular basement membranes. The extent of mesangial and GBM immunoreactivity for CML, but not pentosidine, correlated with the severity of diabetic glomerulosclerosis. The pattern of CML immunostaining in diabetic glomeruli was different from the less specific AGE accumulation observed in sclerosing glomerular diseases including idiopathic and secondary focal segmental glomerulosclerosis and hypertensive nephrosclerosis. Horie et al. (322) demonstrated colocalization of the immunohistochemical expression of CML and pentosidine with a marker of lipid peroxidation (malondialdehyde) in the human kidneys with diabetic glomerulosclerosis. These findings were interpreted as suggestive of local oxidative stress playing a role in the pathogenesis of diabetic glomerular lesions. AGEs have also been demonstrated in the epithelial cells of the proximal tubules both in normal and diabetic kidneys (323). This is likely the reflection of clearance of AGEs by the kidney. Under physiologic conditions, circulating AGEs are cleared from the plasma by glomerular filtration with subsequent reabsorption and metabolism by the proximal tubules (324).

A few human studies have been performed to test the effects of pharmacologic intervention to reduce the deleterious effects of AGEs, with limited success. In two double-blinded placebo-controlled randomized clinical trials, patients with type 1 or type 2 diabetes and overt nephropathy were treated with aminoguanidine, a drug that inhibits AGE formation (325). Aminoguanidine reduced urinary protein excretion; however, the trial was terminated due to side effects including hepatic toxicity and apparent lack of efficacy to prevent diabetic nephropathy.

Strong experimental evidence also exists for the role of AGEs in the development of diabetic nephropathy. Colocalization of AGEs and RAGE has been shown in the glomeruli of STZ-induced diabetic rats (326). It has also been demonstrated that intake of food-derived AGEs contributes to diabetic nephropathy in nonobese (NOB) diabetic and db/db mice, models of type 1 and type 2 diabetes, respectively, and that low AGE diet provides renoprotection (327). Deposition of AGEs in diabetic kidneys may be facilitated by altered expression of AGE receptors that mediate ingestion and degradation of AGEs. Reduced expression of the gene for AGE-R1, an AGE-binding and endocytosis-mediating protein, has been shown in the renal cortex and the mesangial cells of NOB diabetic mice (328). The altered expression of this gene may contribute to delayed AGEs removal and early AGEs deposition in kidney. Increased expression of RAGE, another receptor for AGEs and also receptor for S100/calgranulins, has been demonstrated by podocytes, but not by mesangial cells, of genetically diabetic db/db mice (329). Increased expression of RAGE in this model has been accompanied by mesangial accumulation of AGEs, by the signal transduction ligands for RAGE, and by increased expression of S100/calgranulins, mostly on macrophages. Furthermore, it has been shown that increased expression of VEGF by podocytes was RAGE dependent, a process mediated by S100/calgranulins on inflammatory cells accumulated in the glomeruli. Consistent with these findings, diabetic homozygous RAGE null mice failed to develop significantly increased mesangial matrix expansion or thickening of the GBM. The authors of this study proposed that increased expression and activation of RAGE contribute to podocytic expression of VEGF and enhanced attraction/activation of inflammatory cells in the diabetic glomerulus, thereby setting the stage for mesangial activation and TGF-β production. These processes converge to cause albuminuria and glomerulosclerosis. Another line of evidence pointing to the major role of the AGE-RAGE axis in the development of diabetic glomerular lesions comes from studies with transgenic animals. Diabetic transgenic mice overexpressing human RAGE revealed more prominent mesangial expansion and glomerulosclerosis and increased albuminuria and serum creatinine compared with diabetic littermates lacking the RAGE transgene (330). Experimental data from RAGE knockout animals with STZ-induced diabetes indicate that RAGE is a key modulator of angiotensin II type 2 receptor expression (331). The findings of this study suggest that blockage of RAGE may exert its renoprotective effects via induction of the angiotensin II type 2 receptor. It has also been shown that the GBM in the STZ-treated diabetic rat is resistant to proteolysis (332), a finding suggesting a possible role for AGEs in the pathogenesis of increased thickness of the GBM.

Several experimental animal studies addressed the effects of dietary treatment with aminoguanidine, a substance that interferes with the formation of reactive intermediate glycosylation products (168,295,301,333–335). Brownlee et al. (301) found reduced AGE in rats treated with aminoguanidine as well as reduced numbers of cross-links within components of the GBM accompanied by less thickening of the GBM. Nicholls and Mandel (336) found decreased AGE in diabetic mice treated with aminoguanidine in the face of hyperglycemia. Aminoguanidine also reduced UAE in these models (168,335). In STZ-induced diabetic (mREN-2)27 transgenic rats, a model of diabetic renal disease with an overreactive RAS, aminoguanidine treatment ameliorated glomerulosclerosis and decreased glomerular AGE immunolabeling but had no effect on kidney weight, GFR, or UAE (333). In addition to drugs that inhibit AGE formation or break the AGE cross-links, signal transduction through RAGE can also be inhibited by antisense oligonucleotides, RAGE antibodies, or soluble RAGE. In db/db obese type 2 diabetic mice, treatment with a neutralizing antibody against RAGE for 2 months reduced the increase in kidney weight, glomerular volume, mesangial volume, and UAE, whereas the increase in creatinine clearance and basement membrane thickness was normalized (337). These effects took place without impact on body weight, blood glucose, insulin levels, or food consumption. Renoprotective effects have also been described in STZ-induced diabetic (mREN-2)27 transgenic rats following a 12-week treatment with an AGE cross-link breaker (ALT-946) (333). The treatment ameliorated glomerulosclerosis and reduced cortical tubular degeneration, UAE, and glomerular AGE staining, but had no effect on kidney weight or GFR.

Protein Kinase C Activation Triggered by Hyperglycemia

In addition to hyperfiltration and metabolic and hemodynamic factors, activation of the diacylglycerol-protein kinase C (DAG-PKC) pathway is considered to be one of the main mediators of hyperglycemia-induced tissue injury. Activation of PKC subsequent to the increased de novo synthesis of DAG leads to increased production of transcription factors, blood flow abnormalities, increased vascular permeability, increased production of ROS, ECM synthesis, gene expression, cell growth, differentiation, and angiogenesis (338–341). Increased activity of the polyol pathway as well as AGE-RAGE interaction can also lead to PKC activation (342). Increased activity of PKC, especially PKCβ, has been demonstrated in association with hyperglycemia both in vitro and in vivo. Hyperglycemia-induced activation of PKC isoforms has been shown in glomerular mesangial and endothelial cells cultures (343,344). PDGF induces de novo synthesis of $PKC\beta_{11}$ and activation of a number of PKC isoforms in cultured mesangial cells (345). In cultured mesangial cells, inhibitors of PKCβ isoforms prevented the glycated albumin-induced increased expression of collagen IV (338).

PKC is activated in the glomerulus early in the course of experimental diabetes in rats (346). Increased glomerular PKC activity has also been documented in db/db mice compared with nondiabetic controls (347). In these settings, PKC activation has been shown to mediate renal blood flow abnormalities, perhaps by depressing nitric oxide production and/or increasing endothelin production (348). Activation of mesangial cells mediated by PKC may also contribute to the morphologic manifestations of diabetic nephropathy through increased synthesis of matrix substances such as fibronectin and collagen type IV (349), increased expression of TGF-β1 (349), stimulation of ERK (350), increased NF-κB–dependent cellular proliferation (349), and suppression of MAPK phosphatase-1 (351). Ishii et al. (348), using a specific inhibitor of PKCβ2 in diabetic rats, demonstrated reduction in the GFR and decreased UAE. The beneficial effects of selective PKCβ inhibitors provide further evidence for the relevance of PKC activation in the development of diabetic nephropathy.

Pharmacologic blockage of the PKCβ has been associated with amelioration of accelerated mesangial expansion and expression of genes such as TGF-β and ECM components in diabetic rats and db/db mice (347,349).

Oxidative Stress–Mediated Injury

Compelling evidence supports an important role for ROS in the pathogenesis of diabetic nephropathy. Increased oxidant production secondary to hyperglycemia is thought to be mediated by multiple mechanisms, among which (a) superoxide production by glycolysis and mitochondrial oxidative phosphorylation and (b) receptor-stimulated activation of NADPH oxidase seem to be the most important (352). Experimental evidence suggests that both of these mechanisms have relevance in the pathogenesis of diabetic nephropathy (353). Increased intracellular generation of ROS inactivates NO and activates various redox-sensitive signaling pathways, leading to up-regulation of a number of genes and proteins involved in ECM deposition (353). Activation of some of these genes, such as TGF-β, stimulates ECM production, while others, such as PAI-1, inhibit ECM degradation (354). Brownlee (352) proposed that hyperglycemia-induced overproduction of superoxide by mitochondrial electron transfer chains may play a central role in the development of diabetic complications. Central to this hypothesis is the fact that ROS can activate all major pathogenetic mechanisms (i.e., increased production of intracellular AGEs, increased glucose fluxes through the polyol and hexosamine pathways, and activation of the PKC pathway) involved in the development of microvascular diabetic complications. The effects of oxidative stress are mediated, at least in part, by activating the nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP), which results in inhibition of glyceraldehyde-3-phosphate dehydrogenase-1 (GAPDH) leading to acute endothelial dysfunction as shown in experimental models of diabetes and also in humans with type 2 diabetes (355–357).

Several studies, both experimental and clinical, tested the efficacy of various antioxidants such as vitamins C and E, lipoic acid, antioxidative enzymes, taurine, and others to prevent or delay the onset of diabetic renal complications. The findings of these studies lend some support for the relevance of oxidative stress in the pathogenesis of diabetic nephropathy; however, the findings are not conclusive. Supra-antioxidant doses of vitamin E in STZ-induced diabetic rats normalized the elevated GFR and filtration fraction and also improved UAE (358). However, daily vitamin E treatment for an average of 4.5 years in the Heart Outcomes Prevention Evaluation (HOPE) study that included 3654 people with diabetes showed no effects on the development of new-onset microalbuminuria or overt diabetic nephropathy (359). Short-term combined treatment with vitamin C and E in a double-blind randomized study in patients with type 2 diabetes lowered UAE with no effects on serum creatinine or blood pressure (360).

The Role of Transforming Growth Factor–β and other Growth Factors

Several growth factors have been implicated in the pathogenesis of diabetic nephropathy. We discuss transforming growth factor–β (TGF-β), bone morphogenetic protein-7 (BMP-7), CTGF, VEGF, and PDGF and the growth hormone (GH)-insulin-like growth factor (IGF-I) axis.

Transforming Growth Factor–β1 Several lines of evidence implicate a major role of TGF-β1, a prototype of the TGF-β superfamily, in the pathogenesis of diabetic nephropathy (318,361–363). TGF-β1 exists in a dormant form within the ECM complexed with TGF-β1–binding proteins, and both are cross-linked with matrix proteins by transglutaminase (364,365). Upon generation of an active free form of TGF-β1 followed by binding to a type II serine/threonine kinase receptor, type I receptor is activated. Downstream signaling of Smad, MAPK, and protein kinase A leads to altered expression of genes such as *collagenα1(I)*, *PAI-1, Jun B, c-Jun*, and *fibronectin* (277,364). In addition to AGEs, TGF-β1 signaling is also triggered by ROS, DAG, PKC, and the hexosamines, all of which are generated in excess under high glucose ambience. Increased matrix production coupled with inhibition of MMPs and the activation of tissue inhibitors of MMPs (TIMPs) results in excess ECM deposition. In vitro data show that hyperglycemia up-regulates TGF-β1 in mesangial and glomerular endothelial cells. This can induce the expression of ECM proteins, inhibit MMP synthesis, and stimulate metalloproteinase inhibitor production leading to extracellular (mesangial) matrix accumulation. Several in vivo experimental studies also support the hypothesis that TGF-β1 is responsible for some of the changes observed in diabetic nephropathy. Sharma and Ziyadeh (366) examined the expression of the TGF-β1 gene and protein in the BB diabetic rat and the NOB diabetic mouse. These investigators found that both the protein and the gene were increased in the kidneys of each model in association with renal hypertrophy. These changes in TGF-β1 expression are accompanied by increased production of laminin, tenascin (367), and type IV collagen (316). Bertoluci et al. (368) showed that these changes persisted for ≥24 weeks and were correlated with the appearance of microalbuminuria and morphologic alterations in the kidney. Sharma et al. (369) demonstrated that neutralization of TGF-β1 by specific antibody abrogated the renal hypertrophy and the expression of matrix structural mRNAs. One of the strongest pieces of evidence so far for the significant role of TGF-β1 in the development of diabetic nephropathy has been presented by Ziyadeh et al. (370). In this study, long-term treatment of db/db mice with blocking antibody to TGF-β1 suppressed excess matrix gene expression and thereby glomerular matrix expansion and prevented the development of renal insufficiency in the absence of significant modulation of albuminuria. BMP-7, a member of the TGF-β superfamily, acts in opposition to the profibrogenic effects of TGF-β1 (371). The protective role of BMP-7 in diabetic nephropathy has been demonstrated in transgenic mice overexpressing podocyte BMP-7 with STZ-induced diabetes featuring lesser degree of glomerulosclerosis and tubulointerstitial fibrosis (372). These mice showed reduced renal expression of collagen and fibronectin, better preservation of podocytes, reduced urinary protein, and decreased serum creatinine levels. These findings suggest a potential imbalance in the activity of various members of the TGF-β family in the development of diabetic nephropathy. Imbalance between ECM synthesis and degradation by MMPs and TIMPs can also contribute to the accumulation of ECM proteins. This is corroborated by in vivo experimental data showing decreased glomerular mRNA levels of matrix metalloproteinase-2 (MMP-2) and MMP-3 and increased levels of TIMP in rats with STZ-induced diabetes (373). These findings indicate a complex interplay between

profibrotic cytokines and MMPs and TIMPs in the development of diabetic nephropathy.

Connective Tissue Growth Factor CTGF is another fibrogenic cytokine and is recognized as a downstream mediator of TGF-β1 in renal fibrosis. In vitro studies using dermal fibroblasts and mesangial cells revealed CTGF promoting ECM production, cell adhesion, and collagen matrix contraction (374,375). Another in vitro study showed that CTGF and integrin-linked kinase (ILK) were involved in high glucose–induced phenotypic alterations of podocytes and ILK acted as a downstream kinase of CTGF (376). Significantly elevated levels of CTGF found in the urine of normoalbuminuric juveniles with type 1 diabetes point to the potential role of CTGF in early diabetic renal injury (377). In vitro and in vivo experimental studies by Nguyen et al. (378) presented evidence for a cross-talk between CTGF and BMP-7. The study showed up-regulation of CTGF in diabetic kidneys with inhibition of BMP-7 signal transduction, reduced MMP activity, GBM thickening, and albuminuria, all of which are hallmarks of diabetic nephropathy. The same group of researchers also studied immunohistochemical expression of BMP signaling activity along with two extracellular BMP antagonists, CTGF, and the sclerostin domain-containing-1 (SOSTDC1) in diabetic and nondiabetic patients and also in control and diabetic CTGF (+/+) and CTGF (+/–) mice (379). The main findings of this study include diminished BMP signaling activity (i.e., pSmad 1/5/8 nuclear positivity) and up-regulation of CTGF and down-regulation of SOSTDC1 expression by podocytes in the diabetic patients. CTGF was only expressed in the diabetic wild-type mice but not in CTGF (+/–) mice. pSmad1/5/8 immunohistochemical staining was decreased only in diabetic wild-type mice, but preserved in diabetic CTGF (+/–) mice. The study concluded that overexpression of CTGF, rather than of SOSTDC1, might be an important determinant of loss of BMP signaling and of decreased podocyte number and differentiation in human diabetic nephropathy. Gremlin (grem1) is an antagonist of the BMP family that plays a key role in kidney development (380). There is a growing body of evidence indicating that grem1 might be a potential modifier of renal disease in diabetic nephropathy. Its expression is significantly increased in STZ-induced diabetes in rat kidneys (381) and also in human kidneys with diabetic nephropathy where it colocalizes with TGF-β (382). Mouse mesangial cells exposed to high glucose revealed elevated expression of grem1, increase in cell proliferation, accumulation of the ECM, and enhanced activation of the ERK1/2 pathway (383).

Vascular Endothelial Growth Factor VEGF is a multifunctional glycoprotein with a variety of effects on angiogenesis, endothelial cell proliferation and differentiation, vascular permeability, endothelium-dependent vasodilation, and leukocyte kinetics (318,384,385). VEGF production is regulated by several growth factors and cytokines, including TGF-β, PDGF, and IGF (386). There are compelling in vitro data suggesting a possible pathogenetic role of VEGF in diabetic nephropathy (318,387,388). In rat mesangial cell cultures, high glucose induces VEGF production (389). In cultured human mesangial cells, VEGF has been shown to induce proliferation (390) and increased collagen synthesis (391). In STZ-diabetic rats, increased expression of VEGF has been demonstrated on the podocytes, distal tubules, and collecting ducts after 3 weeks of diabetes (392). Increased expression of VEGF by podocytes in early diabetes exerts its effects by an autocrine and paracrine fashion on the podocytes as well as by endothelial cells and also contributes to the recruitment of macrophages (385). Experimental podocyte-specific overexpression of VEGF in adult mice has been shown to replicate several aspects of diabetic kidney disease featuring glomerulomegaly, GBM thickening, mesangial expansion, loss of slit diaphragms, and podocyte foot process effacement (393). Furthermore, in animal models, inhibition of VEGF ameliorated various diabetes-associated renal changes, such as increases in GFR, glomerular hypertrophy, expansion of the mesangial volume, and UAE (394,395). However, in the human kidney with well-developed diabetes, VEGF expression seems to be lower or absent (396). A recent in vitro study also showed decreased VEGF expression and aberrant expression of ECM proteins by the endothelial cells isolated from the kidney of diabetic mice after only a short duration of diabetes (397). The study concluded that diminished VEGF expression might contribute to the microvascular dysfunction of diabetes. Evidence also exists indicating that angiopoietins are important in maintaining the integrity of the glomerular filtration barrier and that they exert their physiologic effects in a close interplay with VEGF (398). Ang-1, constitutively expressed by the podocytes of a fully developed kidney, promotes endothelial cell survival, while Ang-2 is a competitive inhibitor of Ang-1 (398). Ang-1 exerts its effects via autocrine/paracrine action with its receptor (Tie-2) expressed primarily on endothelial cells and podocytes (399). In both experimental models and humans, dysregulation of Ang-1 and Ang-2 expression in diabetic kidneys has been associated with a decreased Ang-1–to–Ang-2 ratio (400,401). The functional consequences of this seem to be tightly linked to the concomitant VEGF changes in the kidney.

Platelet-Derived Growth Factor The members of the PDGF family exert their paracrine or autocrine effects by activating two tyrosine kinase receptors, PDGF receptor–α, and PDGF receptor–β (318). PDGF is a strong mitogen with effects on chemotaxis, blood vessel tone, platelet aggregation, and tissue homeostasis. In vitro evidence indicates the relevance of the PDGF-PDGF receptor axis in diabetic nephropathy. High glucose increases of both PDGF-B chain and PDGF-β receptor mRNA expression in cultured human mesangial cells (402) and PDGF-β receptor expression in human capillary endothelial cells (403). In mesangial cell cultures, production of collagen induced by AGEs has been shown to be mediated via PDGF (404,405). Also, in mesangial cell cultures, PDGF activates specific PKC isoforms (345) and induces mesangial cell proliferation and migration (406). Experimental animal studies have demonstrated increased immunohistochemical expression of PDGF-B chain and PDGF-β receptor in glomerular podocytes and mesangial cells in short-term STZ-diabetic rats (407). In long-term STZ-diabetic rats, increased expression of glomerular and tubulointerstitial PDGF-B chain has been detected (408,409). In kidney biopsies of patients with type 2 diabetes and overt diabetic nephropathy, gene expression studies revealed a significant up-regulation of PDGF-A and PDGF-B chain mRNA (410). Immunohistochemical analysis showed PDGF-A chain expression in the glomerular and tubular epithelial cells, while PDGF-B chain was predominantly extracellular, located to the

areas of peritubular, interstitial, and periglomerular fibrosis (410). The findings of this study were interpreted as suggestive of a potential role for PDGF in the development of progressive fibrosis of diabetic nephropathy. Using transgenic and knockout mice, deletion of PDGF-β receptor signaling improved diabetic nephropathy in conjunction with decrease in oxidative stress (411). Pharmacologic blockade of PDGF by Trapidil, a nonspecific PDGF inhibitor, has been shown to prevent glomerular hypertrophy in STZ-induced diabetic rats (407).

Growth Hormone-Insulin-Like Growth Factor Axis The growth hormone/insulin-like growth factor (GH/IGF) axis exerts its effects through a complex system with several peptides, including GH, IGF-I, receptors, and binding proteins (318,412,413). Insulin-like growth factor production is induced by GH through specific GH receptors. Mesangial cells express IGF-I receptors and also synthesize IGF-I (318). In vitro data indicate that IGF-I is a potent mitogen for mesangial cells and also stimulates the production of proteoglycan, laminin, fibronectin, and type IV collagen in mesangial cells, promoting ECM accumulation (318). As described earlier, the early changes in diabetic nephropathy include glomerular hyperfiltration and renal hypertrophy. IGF-I has been shown to play a central role in this early growth (240–243). New et al. (242) showed that hyperglycemia increased IGF-I, which was associated with increased renal growth. This response was not abrogated by ACE inhibitor. Furthermore, infusion of IGF-I into normal rats causes renal enlargement (242). In addition, transgenic mice chronically expressing GH or IGF-I exhibit glomerular enlargement (414). Segev et al. (243), using a mouse model of type 2 diabetes, demonstrated that both IGF-I and IGF-binding proteins were increased in the renal cortex. However, growth hormone seems to be also necessary for this early growth (368,415). Flyvbjerg et al. (241) examined dwarf rats with diabetes induced by STZ and found that these rats had low circulating levels of growth hormone and corresponding reduced circulating and tissue levels of IGF. In addition, these dwarf rats showed a slower progression of hypertrophy and other changes of diabetic nephropathy. There is also in vitro evidence indicating that glomerular endothelial NO production is mediated by IGF-I and VEGF (244), raising the possibility that glomerular hyperfiltration in the early stage of diabetic nephropathy is, at least in part, mediated by IGF-I.

The IGF-I signaling pathway has been implicated in the development and progression of diabetic nephropathy with increased mesangial matrix deposition (416,417). In cell cultures, mesangial cells isolated from diabetic NOB mice after the onset of diabetes reveal increased expression of IGF-I and decreased MMP-2 activity (418,419). It has also been shown that excess IGF-I secretion by mesangial cells could contribute to ECM deposition through a decrease of MMP-2 activity (419). Autocrine activation of the IGF-I signaling pathway by constitutively overexpressed IGF-I in the mesangial cells of diabetic NOB mice may lead to decreased ECM degradation (417). Pugliese et al. (420) studied the effects of high glucose in the medium of human mesangial cells grown in vitro. These investigators found that IGF activity was increased, including the number of specific IGF receptors and binding to those receptors. These changes were paralleled by increases in TGF-β transcripts and matrix production with decreased cell proliferation. The authors concluded that increased IGF activity induced by high glucose concentrations could contribute to the pathologic changes of diabetic nephropathy. Additional studies by the same group (421) compared the effects of IGF to TGF-β on cultured mesangial cells. Both growth factors increased the production of fibronectin, laminin, and type IV collagen, although TGF-β was more potent. When both growth factors were added to the culture, the effect was additive, a finding indicating that IGF can stimulate matrix production directly. However, TGF-β has been shown to be a key mediator of increased matrix production in diabetes (422).

Growth hormone levels are increased in diabetic patients. Press et al. (423) showed a relationship between elevation in the level of growth hormone and severity of the metabolic derangements in diabetes. Continuous infusion of octreotide, an analog of somatostatin, reduced GFR and kidney size in a group of patients with type 1 diabetes (424). To address the role of GH in the development of diabetic nephropathy, Esposito et al. (415) examined a model of STZ-treated C57B1/SJL mice that develop glomerular lesions similar to human diabetic nephropathy. Transgenic mice with a mutated form of growth hormone that were made diabetic with STZ treatment were compared with diabetic mice with normal growth hormone. The nontransgenic diabetic mice had increased laminin and type IV collagen, but this increase was not present in the mice with mutated growth hormone. Both types of mice had increase in gelatinase, tissue inhibitor of metalloproteinase, and TGF-β mRNAs induced by hyperglycemia. However, these last three substances were not sufficient to produce diabetic nephropathy in the absence of growth hormone. In other words, an intact growth hormone axis is required for the development of diabetic renal disease.

The Role of Inflammation and Tubulointerstitial Injury

More and more data support the notion that inflammation and tubulointerstitial injury play an important role in the pathogenesis of diabetic nephropathy (425,426). This is in concordance with the pathologic changes of advanced diabetic nephropathy featuring not only glomerular and vascular changes but also interstitial fibrosis, tubular atrophy, and interstitial inflammation. It is well known that IFTA correlate better with renal dysfunction than the glomerular changes both in diabetes and in various forms of glomerular diseases (427). Furthermore, the amount of tubular epithelial cells with phenotypical features of epithelial-mesenchymal transdifferentiation is associated with changes in serum creatinine (428). These observations underlie the significance of the tubulointerstitial compartment in maintaining the functional integrity of the kidney. During the early stages of diabetic nephropathy featuring glomerular hyperfiltration and glomerulomegaly, significant tubular hypertrophy is also present, and it accounts for the vast majority of renomegaly. Strong evidence links tubular hypertrophy to later development of tubular atrophy and renal functional impairment. Furthermore, interstitial inflammation, a common finding in diabetic nephropathy, plays an active role in the progression of tubulointerstitial injury. Some of the pathogenetic mechanisms that contribute to the development of glomerular changes, such as glucose flux to the polyol pathway, generation of AGEs and ROS, and downstream triggering of RAS, PKC, and TGF-β signaling, are also active in mediating tubulointerstitial injury (429–431). Others, like the effects of proteinuria, hypoxemia, and inflammation, seem to be more significant in promoting

tubulointerstitial injury especially during the later stages of diabetic nephropathy (432–436). Proteinuria can mediate tubulointerstitial injury by various mechanisms (277,437), such as via high content of profibrogenic cytokines (433,438), excessive protein reabsorption by the tubular epithelial cells, induction of tubular epithelial-mesenchymal transdifferentiation (439,440), and activation of the complement system (432,441). Complement activation can trigger inflammatory cell influx mediated by MCP-1, further augmenting tubulointerstitial injury (442). The significance of inflammation in diabetic nephropathy is also supported by renal biopsy studies documenting macrophage accumulation as being predictive of declining renal function (443,444).

Chronic hypoxemia due to vasoconstriction or structural abnormalities of the vasculature such as arteriosclerosis or loss of the interstitial capillaries can lead to chronic ischemic injury within the tubulointerstitial compartment (434–436). Hypoxic injury can also develop secondary to capillary endothelial dysfunction mediated by oxidative stress in the diabetic microenvironment (266,445). Impaired mitochondrial function due to hypoxemia might be a significant contributor to apoptotic tubular epithelial cell loss observed in human diabetic nephropathy (130). Hypoxia can also promote epithelial-mesenchymal transdifferentiation and activation of the interstitial fibroblasts (436,446) with ensuing increase in ECM deposition. Hypoxia-inducible factor (HIF-1), a versatile transcription factor, seems to play an important role in this process by regulating the activity of TIMP-1 and CTGF and, thus, promoting fibrosis (447,448).

Endothelial Dysfunction and Structural Abnormalities

There is evidence of widespread endothelial damage and dysfunction in diabetes. The term endothelial dysfunction encompasses a number of abnormalities including disturbances in the barrier function of the endothelium, impaired antithrombotic properties, and perturbed synthetic functions (449). Albuminuria in patients with diabetic nephropathy has been viewed as a sign of global vascular-endothelial dysfunction (70). Elevated serum levels of TM, a marker of endothelial injury, have been documented in experimental animals with diabetes and also in diabetic patients (450–453). In patients with incipient or overt diabetic nephropathy who present with microalbuminuria or macroalbuminuria, significant increases of von Willebrand factor serum levels have been observed compared with diabetic patients without microalbuminuria (451). Another study showed that in patients with type 2 diabetes and macroalbuminuria, serum levels of soluble TM were significantly increased compared with those from patients with the normo-, micro-, or macroalbuminuric stage of diabetic nephropathy without renal dysfunction (454). The increase of soluble TM in sera paralleled levels of urinary albumin, BUN, serum creatinine, and clinical duration of diabetes. Furthermore, a decrease of TM staining in the glomerular capillary walls was observed in both microalbuminuric and macroalbuminuric stages. Recent experimental evidence indicates a protective role of TM in the development of diabetic nephropathy via down-regulating complement activation (455).

Endothelial dysfunction in diabetes may result from complex pathogenetic mechanisms. Decreased bioavailability of nitric oxide (NO) has been proposed to be the central player in this process (456,457). Decreased availability of NO may result from scavenging of NO by glucose and by AGEs and also from activation of PAI-1 (456). Several lines of experimental evidence, both in vitro and in vivo, also support the notion that glomerular endothelial cells, along with mesangial cells, podocytes, tubular epithelial cells, interstitial cells, and vascular endothelial cells, are directly affected by hyperglycemic injury. Hyperglycemia-induced activation of PKC may contribute to altered blood flow and capillary permeability via decreased expression of endothelial NO synthase and NO and up-regulation of endothelin 1 and VEGF, as shown in studies of endothelial NO synthase knockout mice (341,458). Hyperglycemia-induced endothelial cell injury may also lead to the production of special forms of ROS, known as reactive nitrogen species, leading to increased generation of O_2^- (459). Increased oxidative stress may further exacerbate endothelial dysfunction and may lead, along with decreased NO production, to hypoxemia in the tubulointerstitial compartment (460).

The fenestrated glomerular endothelium is one of the three main structural components of the glomerular filtration barrier. Podocyte loss is considered to be a significant contributor to the pathogenesis of diabetic nephropathy. The role of podocyte-derived VEGF in maintaining the structural integrity of the endothelium, including endothelial fenestration, has been well documented (461,462). However, recent morphologic studies demonstrating loss of glomerular endothelial fenestrations in both type 1 and type 2 diabetic patients (159,161) support the notion of a more active role of the endothelial cells in the development of diabetic nephropathy. The functional significance of these structural changes is supported by correlation of reduced GFR with loss of endothelial fenestration in type 2 diabetic patients and by correlation of proteinuria with loss of endothelial fenestration in type 1 diabetic patients (159,161). Whether the changes in glomerular endothelial morphology are related to altered podocyte-derived VEGF bioavailability awaits further investigation.

Systemic Hypertension

The renoprotective effects of systemic blood pressure reduction in diabetic patients are well documented (463,464). Treatment with ACE inhibitors or angiotensin receptor blockers (ARBs) confers independent benefits for rate of loss of renal function, time to ESRD, stabilization of microalbuminuria, regression of microalbuminuria to normoalbuminuria, and mortality for any given blood pressure (465–467). Prospective cohort studies in normoalbuminuric patients with type 1 and type 2 diabetes of 4- to 10-year duration indicated that elevated blood pressure, along with hyperfiltration, poor glycemic control, retinopathy, and smoking, contributes to the development of persistent microalbuminuria and overt diabetic nephropathy (19,468–471). The presence of hypertension and also the visit-to-visit variability of blood pressure are risk factors for the development and progression of diabetic nephropathy in patients with type 2 diabetes (472). Lowering blood pressure in patients with type 2 diabetes provides renoprotection (473). The impact of hypertension on the progression of diabetic nephropathy was further discussed in the clinical presentation part of this chapter.

Experimental (animal) studies also confirm the role of hypertension in hastening the development of diabetic nephropathy. One of the earliest studies was performed by Mauer et al. (474) using the two-kidney one-clip model of hypertension in

diabetic rats. Diabetic renal lesions in the unclipped kidney developed more rapidly and were more severe than in diabetic rats without hypertension. Hemodynamic changes induced by the clip hypertension were postulated as the underlying cause for the differences. Hashimoto et al. (475) showed that diabetic rats lose the ability to autoregulate, so the glomeruli have decreased protection from increases in pressure and flow that accompany hypertension. Insulin therapy in this model reduced the impairment of autoregulation.

Poor Metabolic Control

Several clinical studies have demonstrated that hyperglycemia is an extremely important factor in the initiation and progression of diabetic nephropathy, as discussed in the clinical presentation part of this chapter.

Additional evidence for the potential role of poor metabolic control of blood sugar in the pathogenesis of diabetic nephropathy has come from in vitro studies in which mesangial cells are grown in vitro in high-glucose environments (476–479). In all cases, exposure of the mesangial cells to elevated glucose in the media resulted in increased synthesis of type IV collagen and other matrix components. Takeuchi et al. (479) showed that this production of matrix components was further enhanced by varying the glucose concentration, rather than maintenance of sustained hyperglycemia. Cosio (477) demonstrated that an increase in matrix synthesis was accompanied by inhibition of cellular proliferation. A high glucose content in the media of cultured mesangial cells reduced the activity of the collagenases and other enzymes responsible for degrading matrix (480). This combination of increased synthesis and decreased degradation leads to increased accumulation of matrix substances. Ziyadeh et al. (481) suggested that TGF-β was an important mediator of the effects of glucose on mesangial cells.

A few studies in experimental animals investigated the effects of insulin treatment on the renal lesions. One study using continuous subcutaneous insulin infusion failed to demonstrate a beneficial effect on glomerular lesions in rats (482). However, Petersen et al. (483) compared the effects of 5 months of continuous subcutaneous insulin infusion in rats that had been treated with injections only for the first 6 months of their diabetes with diabetic rats treated with injection only for all 11 months. The group with continuous infusion had decreased UAE and mesangial fraction of glomerular volume when compared with rats receiving injection only. No differences were noted in the thickness of the GBM. Similar results were recorded in a mouse model of type 2 diabetes treated with an oral hypoglycemic drug (484).

Summary of Pathogenesis

The pathogenesis of diabetic nephropathy is complex and involves the interplay of many different factors. A *genetic predisposition* to hypertension is definitely an important factor, although the exact nature of the gene product or functional alteration is not yet known. Moreover, other genetic factors may also play some role. Hemodynamic alterations such as *hyperfiltration* occur in the kidneys of diabetic patients and thus may cause structural injury. Changes in the intrarenal renin-angiotensin axis, alterations in the polyol pathway, changes in prostaglandin synthesis, or increased reactivity to nitric oxide may be directly responsible for the hyperfiltration. The addition of systemic *hypertension* to these hemodynamic alterations worsens the glomerular damage, particularly in combination with impairment of autoregulation, which is commonly present in diabetes. The degree of *metabolic control* affects the development of diabetic glomerulosclerosis. AGEs adversely affect glomerular permeability and the accumulation of macromolecules within the glomerulus. Many of these effects are mediated by transduction of cellular signals by PKC. Finally, the interplay among various *growth factors* clearly contributes to the progression of these lesions.

Clinical Course, Progression, Therapy, and Prognosis

The natural history of diabetic nephropathy in patients with type 1 DM as characterized in the older literature showed variable but steady decline in GFR ranging from 1 to 24 mL/min/yr (mean 12 mL/min/yr) and a concomitant rise in albuminuria and in arterial blood pressure (4,485). Renal function decline can occur prior to the development of microalbuminuria having been noted in as many as 9% of patients with type 1 DM with normoalbuminuria (92). The risk of renal function decline increases to 31% with the presence of microalbuminuria (92). The Steno cohort study showed that 28.5% of 277 patients with type 1 DM progressed from normoalbuminuria to microalbuminuria over a period of 18 years, while 9.7% progressed from microalbuminuria to proteinuria over the same period (90). Reversion to normoalbuminuria reduced the rate of microalbuminuria to 24% at the end of the study (90). Review of additional such studies supported a rate of progression from microalbuminuria to proteinuria at 15% to 30% over 5 to 10 years (486). Skupien et al. (487) followed the rate of renal function decline in 161 patients with type 1 DM for 5 to 19 years. These patients had normal renal function and mild proteinuria (range of albumin creatinine ratio 434 to 1304 mg/g) at enrollment. They found that the slope of eGFR was linear or nearly so in 134 patients. Thirty-six percent of the total had a rate of decline in eGFR of less than 3.5 mL/min/1.73 m^2 resulting in a negligible risk for ESRD. The remaining patients progressed to ESRD most often in a linear fashion determined by the slope of loss of eGFR. This constant rate of loss in individuals with variability of rates of loss between individuals suggests an important genetic component as explanation (487).

Although the great majority of patients with diabetic nephropathy have type 2 DM, far fewer studies and data are available on the natural progression of diabetic nephropathy in patients with type 2 DM (486). In 1996, Nelson et al. (215) published the first study on the natural course of kidney function in patients with type 2 diabetes in Pima Indians followed for 4 years. The GFR was elevated at the onset of type 2 DM, remained elevated while normal albumin excretion or microalbuminuria persisted, and declined progressively after the development of macroalbuminuria. The average decline of GFR was 11 mL/min/yr. Higher renal plasma flow, albuminuria, and body mass index predicted a more rapid decline in GFR, while blood pressure and HbA1c did not. Data from the United Kingdom Prospective Diabetes Study showed progression to microalbuminuria at 2% per year and to macroalbuminuria at 2.8% per year (18). At the outset of this study, 4031 patients did not have microalbuminuria and 5032 had normal renal function. After 15 years of follow-up, 38% of

patients developed microalbuminuria, and 29% had decline in renal function (488). Half of the latter did not have proteinuria. Altemtam et al. (489) examined the decline in eGFR over a period of 8 years in patients with type 2 DM. They found that 34.8% of these patients had progressive decline in eGFR. These progressors could be discriminated from the nonprogressors by higher initial blood pressure, proteinuria, serum uric acid, and lower eGFR (489). Renal artery calcification has also been shown to predict ESRD in patients with type 2 DM (490).

Among patients starting renal replacement therapy, diabetes is the leading cause of ESRD (i.e., kidney failure requiring dialysis or transplantation) in the Western world. In the United States, diabetes accounted for 44.8% of treated new cases of ESRD in 2010 (approximately 152 individuals per million population) although the rate of incident cases has remained stable over the past 10 years (5). Patients who have type 1 DM and proteinuria develop ESRD more often than those who have type 2 DM and proteinuria. The lifetime risk of progression to ESRD in type 1 DM has been shown to be 25% (491). Two groups have demonstrated that 35.5% and 52% of patients with type 1 DM and macroalbuminuria developed ESRD followed for a median of 9.9 and 15 years, respectively (492,493), compared to 3% to 11% in proteinuric type 2 diabetic patients (494). However, due to the higher prevalence of type 2 DM, 80% or more patients with ESRD secondary to diabetes have type 2 DM. The incidence of ESRD is also higher in women reflecting the demographics of diabetes and diabetic nephropathy in the general population.

Pugh et al. (495) compared the course to ESRD in patients with type 1 DM and those with type 2 DM. These investigators found that hyperglycemia was more prominent in the patients with type 1 DM and renal disease, whereas hypertension was more frequently seen in the patients with type 2 DM and renal disease. In agreement with other studies, they found that the course to ESRD was shorter in patients with type 2 DM. They also noted that patients with type 1 DM more frequently suffered from other microvascular complications such as retinopathy or neuropathy, whereas patients with type 2 DM more often had myocardial infarcts or congestive heart failure.

Diabetic Nephropathy After Transplantation

Kidney transplantation is the best therapeutic option for patients with type 2 diabetes with ESRD (496). In patients with type 1 diabetes with ESRD, simultaneous pancreas and kidney transplantation is the preferred modality. However, diabetic nephropathy can recur in the transplanted kidneys (104,497–499). The first such study showed that the earliest change in kidneys from nondiabetic donors when transplanted into patients with diabetes was the appearance of hyaline arteriolosclerosis (499). Ten of the twelve patients studied had such lesions within 5 years of renal transplantation. These lesions did not seem to affect graft function. One patient developed nodular glomerulosclerosis. Only 3 of 28 nondiabetic patients receiving renal allografts showed hyaline arteriolosclerosis, and their lesions affected fewer arterioles than seen in the diabetic patients. Other investigators (497,498) confirmed the early appearance of hyaline arteriolosclerosis and also noted the presence of linear staining of the GBMs for IgG and albumin. Each of these investigators reported only single cases of recurrence of nodular glomerulosclerosis in studies of 17 and 18 patients, respectively. Bohman et al. (497) also noted increased mesangial matrix and thickened GBM in about half of their patients. No correlation was seen between the morphologic changes and degree of metabolic control, blood pressure, or clinical duration of diabetes at time of renal transplantation. These investigators believed that recurrence of diabetic nephropathy in a transplanted kidney occurs more quickly than the 10 to 15 years usually required for the development of diabetic glomerulosclerosis in native kidneys. Mauer et al. (104) examined biopsies of allografts in 18 patients with type 1 diabetes after 6 to 14 years and found that five of the patients had no major morphologic changes, six had mild mesangial expansion, and seven had moderate to marked mesangial widening. The authors found a correlation between the degrees of mesangial expansion and mean plasma glucose. However, because of the variable rate of recurrence of diabetic nephropathy in the transplanted kidneys and the lack of correlation with several potential risk factors, the authors hypothesized that factors intrinsic to the kidney (intrinsic risk factors) may contribute to the development of diabetic nephropathy. In a prospective study, Barbosa et al. (500) examined the effect of glycemic control on the development of diabetic nephropathy in the allograft. Patients were given either standard or maximal insulin therapy and underwent biopsy 5 years after surgery. No difference was noted in the blood pressure of the two groups, and only a slight increase was seen in glycosylated hemoglobin in the group receiving standard therapy. Morphometry revealed a significant increase in the mesangium and the volume fraction of the mesangial matrix with a trend for increase in GBM thickness and in hyaline arteriolosclerosis. The authors concluded that a causal relationship exists between glycemic control and mesangial expansion in this setting. In 1996, Hariharan et al. (501) reported a relatively high rate of graft failure due to recurrent diabetic nephropathy in patients with type 1 diabetes. Recurrent diabetic nephropathy was diagnosed histologically in 14 renal transplant recipients, on average, approximately 8 years after transplantation. The patients presented with proteinuria (mean, 5.3 g/24 hours) and renal dysfunction; hypertension was also common. The most consistent morphologic finding was diffuse GBM thickening with mild mesangial matrix accumulation. Nodular diabetic glomerulosclerosis was only present in 2 out of 14 cases. During the follow-up period, 9 out of 14 grafts were lost with recurrent diabetic nephropathy ($n = 7$) being the most common cause of graft loss. The 1-, 2-, and 3-year graft survival rates after the diagnosis of recurrent diabetic nephropathy were 83%, 55%, and 22%. Kim and Cheigh (502) studied patient and graft survival, graft function, and cause of graft failure in 78 type 1 diabetic kidney transplant recipients compared with 78 nondiabetic patients. Cumulative patient and graft survival rates for diabetic patients were lower than that of nondiabetic patients, but the differences did not reach statistical significance for graft survival. Among the 16 patients who had transplant renal biopsies 2 to 7 years posttransplant, six showed morphologic changes consistent with diabetic nephropathy, but only one graft was lost due to recurrent diabetic nephropathy.

Diabetic nephropathy can also be seen in patients who develop diabetes posttransplant (i.e., new-onset diabetes after transplantation [NODAT]) whose primary disease was not diabetes; diabetic nephropathy in this setting is usually designated

as de novo diabetic nephropathy (503–505). A study by Bhalla et al. (503) showed that the incidence of de novo diabetic nephropathy in patients who develop diabetes posttransplant is similar to the incidence of recurrent diabetic nephropathy in patients whose original renal disease was diabetes. Also, this study documented that mean clinical duration of diabetes at the time of histologic diagnosis of diabetic nephropathy was similar in the de novo and recurrent diabetic nephropathy groups. Diabetic nephropathy, as defined by characteristic morphologic changes of mesangial matrix accumulation, diffuse thickening of the GBMs, arteriolar hyalinization, and pseudolinear immunofluorescence along the glomerular, tubular, and Bowman capsular BMs with IgG and albumin, developed more rapidly in both the de novo and recurrent groups (6.68 ± 3.86 and 5.90 ± 3.13 years, respectively) than that described in an earlier study by Hariharan et al. (501) in patients with recurrent diabetic nephropathy. Interestingly, neither the usual clinical risk factors of diabetic nephropathy, such as poor glycemic control, elevated blood pressure, and hyperlipidemia, nor the clinical variables related to transplantation clearly distinguished the group with diabetic nephropathy from the group without it. There are also data indicating that NODAT is associated with impaired long-term patient and renal allograft survival and function (496,506,507).

Data also document regression of diabetic nephropathy when kidneys from diabetic donors are transplanted into nondiabetic patients (508,509). Four donors for a total of eight kidneys were involved in these studies. Three of the four donors had diabetic glomerulosclerosis. One of the kidneys failed in 3 days because of rejection. The remaining allografts showed a reduction in diabetic lesions shown by biopsy 6 months to 2 years after renal transplantation. Additional evidence confirming the importance of the diabetic environment for the maintenance of diabetic lesions in the kidney comes from the study of Bilous et al. (510). These investigators examined the effects of pancreatic transplantation on renal morphologic features in 12 diabetic patients who had undergone renal transplantation 1 to 7 years previously and compared the results with renal allograft biopsies from diabetic patients without pancreatic transplants. The patients with pancreatic transplants had smaller glomeruli with less mesangial expansion; however, there were no differences in the thickness of the GBM between the two groups. Wilczek et al. (511) compared kidney biopsies from allografts 1 to 6.5 years after transplantation in patients with and without pancreatic transplantation. The patients who received only kidney transplants showed increased basement membrane thickening and relative volume of mesangial tissue. Many of the patients with combined pancreas and kidney transplants had no morphologic alterations in their renal allograft biopsies. Fioretto et al. (512) studied the effects of pancreatic transplantation on diabetic renal lesions compared with a group of diabetic patients who did not receive a transplant. Baseline values and biopsies at 5 years were reviewed in the two groups. Mesangial volume remained stable in the patients with pancreas allografts, but it increased in the patients without such transplants. However, no reduction was noted in the changes that were present at baseline. Fioretto and Mauer (513) studied renal structure before and 5 and 10 years after pancreas transplantation in eight type 1 diabetic patients. These patients had established diabetic nephropathy at the time of pancreas transplantation. Although diabetic glomerulopathy was unchanged at 5 years post–pancreas transplantation, there was a complete normalization of glomerular structure in most patients 10 years posttransplantation.

Islet Transplantation

Numerous authors have shown a beneficial effect of islet transplantation on the glomerular lesions in diabetic rats and mice. Changes have chiefly included reduction in glomerular size (514) or in mesangial expansion (515,516). No decrease occurs in thickness of the GBM (514). Nicholls and Mandel (336) found that islet transplantation prevented the further accumulation of AGE but confirmed no decrease in thickness of the GBM.

Progression Factors and Renoprotection

Because only 30% to 40% of diabetics develop diabetic nephropathy and the occurrence of such lesions decreases life expectancy, it is important to determine predictors of nephropathy as well as means to delay or prevent the progression of this disease. Several factors including onset of type 1 diabetes later in life, parental type 1 diabetes, edema, and an abnormal electrocardiogram independently predict progression of renal disease in diabetes (72). However, the most important factors include hypertension and the degree of metabolic control.

Blood Pressure Control

The high prevalence of hypertension in patients with type 1 diabetes (40%) and type 2 diabetes (70%), even before the onset of microalbuminuria, is well established (517). The impact of hypertension on the progression of diabetic nephropathy also was well documented. Ravid et al. (518) followed 195 patients with type 2 diabetes with normal blood pressure at the outset of the study for 14 years and examined the relationship between their blood pressure and the progression of renal disease. These investigators found that the patients who became hypertensive showed an increased propensity for developing proteinuria (60.0% compared with 20.8% in the normotensive group) despite similar degree of metabolic control. Furthermore, among the patients who developed proteinuria, the hypertensive patients showed a greater decline in the GFR. Parving et al. (519) examined the effects of long-term antihypertensive therapy on progression of diabetic renal disease compared with the natural history of the disease. These investigators studied 45 patients with type 1 diabetes and nephropathy for a median follow-up of 16 years. The cumulative death rate was 45% 16 years after the onset of diabetic nephropathy compared with 94% recorded by Krolewski et al. (16). The latter study was an examination of the natural history of the disease without antihypertensive therapy.

There is a strong association between blood pressure, increase in albuminuria, and the rate of decline in GFR in both type 1 and type 2 diabetic patients (72,73,136,520–525). Early studies showed that long-term antihypertensive treatment in type 1 diabetic patients with nephropathy reduces albuminuria and the rate of decline of GFR (68). This finding has been confirmed by several follow-up studies. The time-dependent renoprotective effects of long-term aggressive antihypertensive treatment in patients with type 1 diabetes are demonstrated in Figure 21.27. Treatment of hypertension also significantly reduces the risk of development of microalbuminuria. In patients with type 2 diabetes, a reduction of systolic

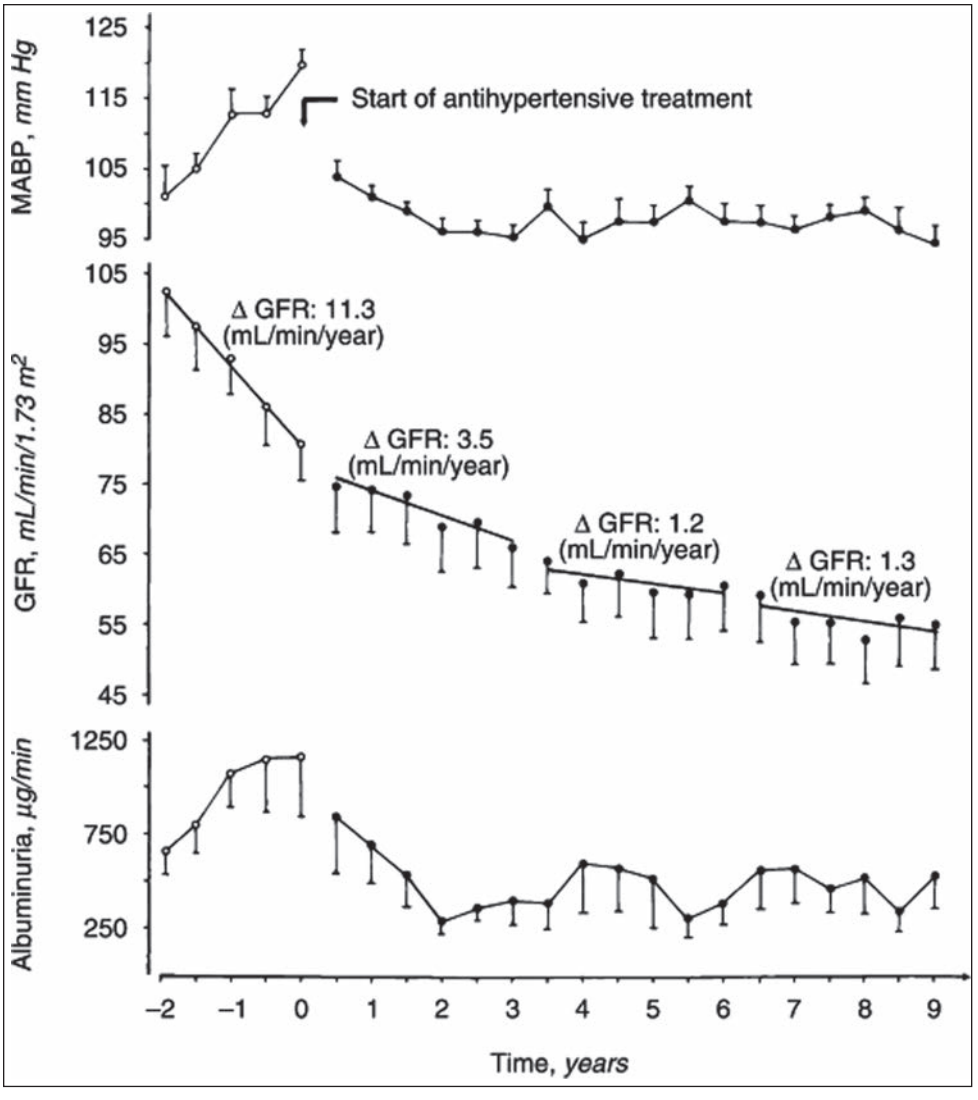


FIGURE 21.27 Average course of mean arterial blood pressure, GFR, and albuminuria before (○) and during (•) long-term effective antihypertensive treatment on 11 patients with type 1 diabetes and nephropathy. The rate of decline in GFR decreased from 10.7 mL/min/yr before treatment to 2.5 mL/min/yr during treatment. During the median pretreatment period of 2.4 years, the GFR decreased significantly, and albuminuria and the arterial blood pressure increased significantly. During the 9.7-year period of antihypertensive treatment with metoprolol, hydralazine, and furosemide, the arterial blood pressure decreased from 143/96 to 130/84 mm Hg, and albuminuria decreased from 1038 µg/min to 547 µg/min. (From Parving HH, Smidt UM, Hommel E, et al. Effective antihypertensive treatment postpones renal insufficiency in diabetic nephropathy. *Am J Kidney Dis* 1993;22:188; with permission.)

blood pressure from 154 to 144 mm Hg reduced the risk of the development of microalbuminuria by 29% (526). Effective control of hypertension has also been shown to slow the rate of progression to renal failure even in patients with overt nephropathy (485,527,528). Sawicki et al. (528) compared patients with long-term diabetes who received either routine antihypertensive therapy or intensive treatment for hypertension. The patients were followed for a mean of 68 months. Four percent of the patients in the intensive treatment group died compared with 28% in the routine treatment group. Renal replacement therapy was required in only 9% of the patients in the intensive therapy group compared with 23% in the routine treatment group, and progression of renal disease occurred in 27% of the intensively treated group compared with 59% of the other group.

Antihypertensive treatment not only slows down the rate of decline of GFR but, in some patients, may lead to regression of the disease (i.e., δ GFR [≤1 mL/min/yr] [equal to the natural aging process]). In a study of 10 type 1 diabetic patients with nephropathy, Parving et al. (527) showed slowing of the rate of decline of the GFR from 0.91 mL/min/mo in a period of 29 months before aggressive antihypertensive treatment to a rate of 0.39 mL/min/mo during an interval of 39 months of aggressive antihypertensive therapy. This change was also accompanied by a decline in UAE. No difference was seen in degree of metabolic control during the two periods of investigation.

Higher blood pressures, even in the range considered normal, are predictive of diabetic nephropathy risk. Patients with advanced diabetic nephropathy and type 1 diabetes had higher mean arterial blood pressure during adolescence (529). The risk of developing nephropathy is also enhanced in diabetic patients with a family history of hypertension (246). Krolewski et al. (11) studied 89 patients approximately 20 years after the onset of type 1 diabetes. Thirty-three of these patients had nephropathy at follow-up. The risk of nephropathy was tripled for those patients having a parent with hypertension. Furthermore, the patients with nephropathy had higher maximal velocity of lithium-sodium countertransport in red cells, a marker of risk for essential hypertension. The risk of developing nephropathy was increased still more in those patients with a history of poor metabolic control in their first 10 years of the disease. The authors of this study concluded that the risk of renal disease in diabetics is increased in patients with a genetic predisposition to hypertension, with a further increment in the risk resulting from poor metabolic control.

In addition to its beneficial effects on the rate of progression of diabetic nephropathy, aggressive blood pressure–lowering therapy also significantly extends the median survival of diabetic patients with persistent proteinuria. Early studies showed an average survival of patients with persistent proteinuria due to diabetic nephropathy of 5 to 7 years (530–532); the renal prognosis is similar in patients with proteinuria who have type 1 or type 2 diabetes (533,534). A significantly improved median survival time of 14 years or greater has been documented in patients treated with aggressive antihypertensive therapy (519,535). Data from the Framingham and the Multiple Risk Factor Intervention Trial Diabetic Cohort showed that cardiovascular mortality was increased by a factor of 2 to 4 in diabetic patients and there was a clear association between systolic blood pressure and complications without any threshold value (536).

Glycemic Control

Studies beginning in 1982 indicated that microalbuminuria in diabetic patients is a good predictor of the later development of diabetic nephropathy and decline in renal function (81,82,537) (Fig. 21.28). Several groups showed that the extent of UAE correlates with the degree of glycemic control both in type 1 diabetes (81,538,539) and in type 2 diabetes (81,537,538,540). This finding led investigators to examine the effect of strict metabolic control during the stage of incipient nephropathy on the later development of overt glomerulosclerosis. The importance of degree of metabolic control in the progression of diabetic renal disease is illustrated in the study of Krolewski et al. from the Joslin Clinic (491). These investigators found that the degree of glycemic control in the first 20 years of type 1 diabetes was a strong predictor of ESRD. The prevalence of ESRD was 36.3% in patients with the worst control compared with 14.4% with better control and only 9.2% in those with the best control. Stephenson et al. (541) found similar results in Europe in a study of 3250 patients with type 1 diabetes.

Bangstad et al. (542) performed a prospective study of patients with type 1 diabetes and microalbuminuria in two groups randomized to conventional treatment or to continuous subcutaneous insulin infusion. Renal biopsies were taken at the beginning of the study and again between 26 and 34 months later. The investigators found that strict metabolic control was established in the infusion-treated group as determined by mean glycosylated hemoglobin levels. The GBM thickness increased in each group, but the increment of GBM change was larger in the conventional therapy group. Volume fraction of the mesangial matrix was increased only in the conventional therapy group. The investigators concluded that a close relationship was present between the level of blood glucose and the characteristic changes of early diabetic nephropathy.

Meta-analysis of several randomized studies (543) comparing the effects of long-term intensive versus conventional blood glucose control on the risk of nephropathy progression in normo- (80%) and microalbuminuric type 1 diabetic patients showed beneficial effects on the progression from normo- to microalbuminuria by intensive treatment. Intensified glycemic control in the Diabetes Control and Complication study did not decrease the rate of progression to macroalbuminuria in patients with type 1 diabetes who were microalbuminuric at the beginning of the study (544,545). Strict glycemic control in patients who were normoalbuminuric at the beginning of the study did, however, reduce the occurrence of microalbuminuria by 39% and that of albuminuria by 54% at the end of the 6.5-year study (544). Also, improved glycemic control reduced the renal functional deterioration in proteinuric patients with type 1 diabetes in another study (546).

Only few studies analyzed the effects of glycemic control on the rate of progression of nephropathy in patients with type 2 diabetes. The progression rate of microalbuminuria to macroalbuminuria was reduced with intensive glycemic control in a Japanese study (547). Progressive beneficial effects of intensive metabolic control have also been found by the UK Prospective Diabetes Study on the development of microalbuminuria and overt proteinuria (548). However, several studies failed to demonstrate a significant correlation between glycemic control and progression of GFR in albuminuric patients with type 2 diabetes (215,517,549,550). However, three trials assessing the effects of intensive glycemic control showed delayed onset or progression of diabetic nephropathy

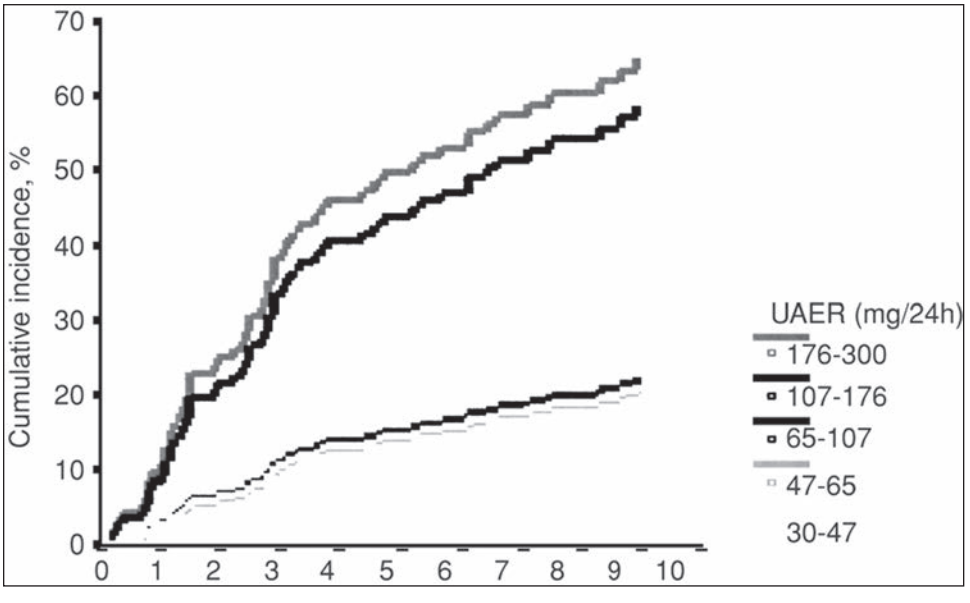


FIGURE 21.28 Cumulative incidence of overt diabetic nephropathy according to quintiles of UAE rate level in 334 microalbuminuric type 1 diabetic patients. During a 10-year follow-up period, the presence of microalbuminuria predicted progression to overt nephropathy in 33% of type 1 diabetic patients, whereas 16% of the patients regressed (not shown) to normoalbuminuria. (From Rossing P, Hougaard P, Parving HH. Progression of microalbuminuria in type 1 diabetes: ten-year prospective observational study. *Kidney Int* 2005;68:1446; with permission.)

in patients with type 2 diabetes (551). A systematic review of studies published between 2003 and 2010 of patients with either type 1 or type 2 diabetes with or without CKD revealed no improvement in clinical outcomes (i.e., all-cause mortality, death from cardiovascular causes, incident kidney failure, and nonfatal cardiovascular events) with intensive glycemic control and lipid interventions (222).

It is now generally accepted that the degree of glycemic control is an important factor in the development and evolution of diabetic nephropathy. The impact of hyperglycemia on progression of diabetic nephropathy has been well documented especially during the early stages of the disease, before albuminuria had developed. The impact of hyperglycemia during the later stages of the disease, once albuminuria has developed, is debated. Furthermore, intense glycemic control has been associated with an increased risk for cardiovascular death in type 2 diabetics (552).

Blockade of the Renin-Angiotensin System

Blockade of the RAS with ACE inhibitors or ARBs confers additional benefit on preserving renal function. The first large study of these agents was reported by Lewis et al. (553), who examined the effect of captopril in a randomized controlled study of 409 patients with type 1 diabetes with a median follow-up of 3 years. These investigators found that doubling of the serum creatinine occurred in 43 of the patients taking the placebo but in only 25 patients who received captopril. The risk of death, or the need for either dialysis or renal transplantation, was reduced by half in the captopril-treated group. These investigators concluded that captopril slowed the progression of diabetic nephropathy more effectively than simple control of blood pressure. Ravid et al. (554) examined 94 patients with type 2 diabetes, serum creatinine less than 1.4 mg/dL, and microalbuminuria between 30 and 300 mg/24 hours. The patients were assigned to treatment with enalapril for 5 years or placebo. Each subject then had a choice of receiving enalapril or receiving no therapy for the next 2 years. The patients who received enalapril therapy for 7 years had stable microalbuminuria during that period. The untreated group showed an increase in microalbuminuria from 123 to 310 mg/24 hours in 5 years, with an additional increase to 393 mg/24 hours in the last 2 years of the study. The treated patients showed no change in the reciprocal of the serum creatinine. Those who did not receive any therapy showed a decline of 13% in 5 years and 16% at 7 years. Treatment resulted in an absolute risk reduction of 42%. Discontinuation of therapy renewed progression of the renal disease. Six of the 33 patients (18%) who received treatment throughout the 7-year period progressed to macroalbuminuria, compared with 12 of 20 patients (60%) who were not treated with enalapril. Mean blood pressure was maintained at ≤108 mm Hg in all patients.

The findings of three randomized, placebo-controlled studies in type 1 and type 2 normotensive and normoalbuminuric diabetic patients have suggested beneficial effects of ACE inhibitor therapy on the development of microalbuminuria (555–557). A meta-analysis of 12 trials evaluating 698 nonhypertensive microalbuminuric patients with type 1 diabetes treated with ACE inhibitors also showed decrease of the risk of progression to proteinuria by 60% and increase of the chances to revert to normoalbuminuria (558). It has also been documented that in type 2 diabetic patients with hypertension and normoalbuminuria, the renoprotective effects of blood pressure reduction were similar in those treated with ACE inhibitors versus calcium antagonists (556,559) or β-blockers (560). Type 2 diabetic patients with microalbuminuria also benefit from treatment with ACE inhibitors. In a double-blind randomized study, type 2 diabetic patients with microalbuminuria and normal blood pressure were receiving enalapril or placebo for 5 years (561). The renal function remained stable, and only 12% of patients in the study group developed diabetic nephropathy. In contrast, the renal function in the placebo-treated group declined by 13% and 42% of patients in this group developed nephropathy. The short-term renoprotective effects of ACE inhibitors were similar to those of ARBs in reducing the albuminuria in patients with type 2 diabetes (562,563). In the MICRO-HOPE study (557), the ACE inhibitor ramipril decreased the risk of overt nephropathy by 24% and the risk of cardiovascular death in patients with type 2 diabetes who were greater than 55 years of age with one additional cardiovascular risk factor by 37%. It has also been documented that dual blockade with ACE inhibitors and ARBs is more beneficial than therapy with ACE inhibitors or ARBs alone (563). The ADVANCE (Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation) study showed that lowering the blood pressure below 125/75 mm Hg in patients with type 2 diabetes and nephropathy provides important renoprotection (564). This study documented a 21% reduction in the risk for renal events during a mean follow-up of 4.3 years ($P < 0.0001$).

The effects of these agents are complex but include alterations in glomerular hemodynamics resulting in decrease of hyperfiltration, inhibition of glomerular and tubular hypertrophy, and lowering of systemic blood pressure (565–567).

Prognosis of Diabetic Nephropathy

The development of proteinuria and renal disease in patients with either type 1 or type 2 DM decreases life expectancy (6,81). Deckert et al. (6) studied the outcome of a group of 289 patients (predominantly those with type 1 diabetes) after ≥40 years of diabetes. Forty percent were alive at the time of follow-up with a mortality rate two to six times that of a control nondiabetic group. The cause of death was uremia in 31% and myocardial infarction in 25%, with excess mortality three to four times higher in the patients with proteinuria (6). Declining mortality rate and incidence of renal failure in DM type 1 have been demonstrated by the Pittsburgh Epidemiology of Childhood Onset Diabetes Complication Study (568). This study followed patients from onset of disease up to 30 years in cohorts of 5 years beginning in 1950 and ending in 1980. All-cause cumulative mortality at 20 years decreased from 22% in the 1950–1959 cohort to 3.5% in the 1975–1980 cohort. At 30 years, a decline from 39% in the 1950's group to 23% in the 1965–1969 cohort was observed. Likewise, renal failure declined at 20 years from 16% for those in the 1950s to 4% for those diagnosed after 1964. At 30 years, renal failure decreased from 31% in the 1950's group to 18% in the 1965–1969 cohort (568). No significant difference was seen in the incidence of overt nephropathy. Finne et al. (8) showed an even lower incidence of ESRD at 30 years of 7.8% for a cohort from 1965 to 1969. Mortality is also higher in type 2 diabetes patients with proteinuria. In this group, 45% died of cardiovascular disease and 5% died as a result of uremia (81).

It has been shown that type 1 diabetics in the highest quartile of hemoglobin A1c have a higher mortality rate and greater incidence of ESRD at 20 years from diagnosis (569) suggesting that better glycemic control might lead to greater longevity and better quality of life. Epidemiologic studies have shown an association between poor glycemic control and incidence of microvascular complications in type 2 DM (570). Similarly intense glycemic control reduces microalbuminuria in type 2 DM (571,572). However, the ACCORD study resulted in an increased risk for cardiovascular death with intense glycemic control in type 2 DM (552). A recent meta-analysis (221) failed to demonstrate a reduction in doubling of serum creatinine, ESRD, or death from renal disease during intense glycemic control. However, Perkovic et al. (573) reported that intensive glycemic control as used in the ADVANCE trial reduced the risk of ESRD and resulted in improvement in albuminuria. Clearly more investigation is required to discern the best treatment to improve prognosis in diabetic nephropathy.

Clinicopathologic Correlation

As discussed in detail in the section on Pathologic Findings the morphologic changes in diabetic nephropathy affect all four renal compartments, that is, glomeruli, tubules, interstitium, and vessels. The changes in each of these four compartments encompass a spectrum of morphologic alterations showing good overall correlation with the biologic duration of the disease. Detailed morphometric studies especially in patients with type 1 diabetes have documented that some of the structural-morphologic changes, including both glomerular and tubulointerstitial changes, show close correlation with the clinical-laboratory parameters of renal dysfunction. Studies addressing the structural-functional correlation in patients with type 2 diabetes have demonstrated, in general, less precise correlation between morphologic findings and renal functional parameters than those seen in patients with type 1 diabetes probably secondary to the heterogeneity seen in type 2 diabetes.

The characteristic glomerular changes in diabetes include glomerular enlargement, diffuse thickening of the glomerular capillary basement membranes, mesangial expansion primarily due to matrix accumulation, and progressive glomerulosclerosis with development of globally sclerotic glomeruli. One of the earliest morphologic changes detected in diabetic nephropathy is the diffuse thickening of the glomerular capillary basement membranes (574). Precise quantitative measurements demonstrated that in patients with type 1 diabetes, thickening of the proximal tubular basement membranes is strongly related to the thickening of the glomerular capillary basement membranes (575). Although mesangial matrix accumulation with mesangial expansion represents one of the most fundamental morphologic changes of diabetic glomerulosclerosis, the increase in the mesangial fractional volume (i.e., the volume fraction of the glomerulus occupied by mesangium) can only be documented 4 to 5 years after the onset of diabetes in patients with type 1 diabetes (574). Increases in the mesangial fractional volume correlate precisely with the decrease in the peripheral glomerular capillary basement membrane filtration surface density (148). A close relation between the mesangial fractional volume and UAE rate in patients with type 1 diabetes has been documented in studies utilizing elegant morphometric analyses (148,576) (Fig. 21.29). In patients with type 1 diabetes, thickness (width) of the glomerular capillary basement membranes directly correlates with the blood pressure and the UAE rate; however, the correlation is weaker than that seen with mesangial fractional volume (148,576). The width of the glomerular capillary basement membranes is inversely correlated with the GFR (576), while total peripheral capillary filtration surface is highly correlated with GFR in patients with type 1 diabetes (151,577). In general, thickening of the glomerular capillary basement membranes and degree of mesangial expansion increase in patients with type 1 diabetes from normoalbuminuria to microalbuminuria to proteinuria; however, considerable overlap exists among these groups (576). Glomerular capillary basement membrane thickening

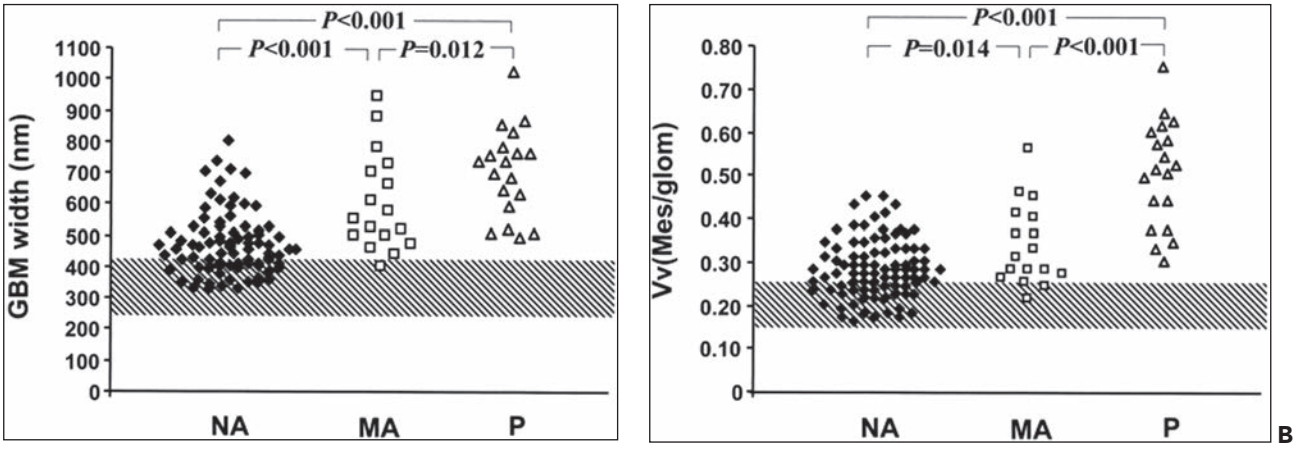


FIGURE 21.29 GBM width (A) and glomerular fractional volume (Vv[Mes/glom]) (B) in 88 normoalbuminuric (NA), 17 microalbuminuric (MA), and 19 proteinuric (P) patients with type 1 diabetes. The *hatched areas* represent the mean ± 2 SD in a group of 76 age-matched normal control subjects. All groups are different from control subjects. (From Caramori ML, Kim Y, Huang C, et al. Cellular basis of diabetic nephropathy. 1. Study design and renal structural-functional relationships in patients with long-standing type 1 diabetes. *Diabetes* 2002;51:506.)

and mesangial expansion can progress at varying rates within and between patients with type 1 diabetes (148,576,578). In some patients, marked glomerular capillary basement membrane thickening can be seen without significant increases in the mesangial fractional volume, while others may show the opposite pattern (148,578). In fact, some normoalbuminuric patients with type 1 diabetes can have advanced glomerular lesions and other clinical findings of advancing renal disease such as low GFR and hypertension (576). This is an indication that albuminuria, albeit a strong predictor of progression, is still an imprecise indicator of diabetic nephropathy risk.

Abnormalities of the endothelial cells and visceral epithelial cells (podocytes) have been described in both type 1 and type 2 diabetic patients. Decreased endothelial cell fenestration has been identified in both type 1 and 2 diabetics and correlates to the presence of albuminuria and other diabetic changes (159,161). A significantly decreased number of podocytes have been detected in both type 1 and type 2 diabetes (103,116–118,579–582). A significant inverse correlation between the number and density of podocytes per glomerulus and proteinuria has been reported in patients with type 2 diabetes (118). In the Pima Indians with type 2 diabetes, the number of podocytes per glomerulus was the strongest predictor of progressive renal disease, with fewer cells predicting more rapid progression (583).The degree of podocyte detachment from GBM correlated directly with the amount of albuminuria in both type 1 and 2 diabetics (159,161). Podocyte detachment has also been described in other proteinuric diseases particularly in areas of segmental sclerosis (584). Adhesions of the glomerular tuft to the Bowman capsule may form following podocyte injury with detachment (106). Such lesions particularly at the glomerulotubular junction have been shown to correlate to the degree of proteinuria in type 1 diabetes and are found almost exclusively in such patients with overt proteinuria (124). Decreased glomerular expression of podocyte-associated nephrin, a protein known to have a significant role in maintaining normal permselectivity of the glomerular filtration barrier, has been described in patients with type 1 and type 2 diabetes (585–587). Decrease in nephrin expression correlated with the broadening of the foot process width. One study indicated that reduction in nephrin expression was due to down-regulation of expression rather than decreased number of podocytes in the glomeruli (585).

Tubulointerstitial changes in diabetic nephropathy include interstitial expansion due to increase in cellular content and/or fibrosis and tubular atrophy. Mild interstitial expansion in patients with long-standing type 1 diabetes is largely due to an increase in interstitial cell component that antedates interstitial fibrosis (135). Increased fractional volume of interstitial fibrillary collagen appears at later stages of the disease, when the GFR is already reduced (135). This is different from the glomerular lesions where mesangial matrix accumulation is the primary cause of mesangial widening even at the early stages of the disease. The extent and/or severity of interstitial fibrosis and global glomerulosclerosis have also been shown to correlate with the clinical manifestations of diabetic nephropathy in patients with type 1 diabetes (137,196). The extent of interstitial fibrosis has long been advocated as an independent predictor of chronic renal dysfunction as characterized by elevated serum creatinine not only in patients with diabetes including those with type 2 diabetes but also in a number of nondiabetic glomerular, tubulointerstitial, and vascular diseases (133,588). In a prospective study, Bangstad et al. (589) compared various morphometric parameters to markers of endothelial activation and inflammation at three different timepoints in patients with type 1 diabetes. They found that PAI-1 activity at baseline (10 years from onset of diabetes) was an independent predictor of interstitial fractional volume 8 years later (589).

In a recent review, Najafian and Mauer (106) state that morphometric techniques are useful predictors of renal functional decline in type 1 diabetes only in later stages and not early in the course of disease. They attribute this to the heterogeneity of the disease within individuals. As a corollary, structural-functional correlations in patients with type 2 diabetes are less conclusive than those in patients with type 1diabetes due to the greater morphologic heterogeneity of the renal lesions in these patients.

Differential Diagnosis

Many features of diabetic nephropathy may occur in other glomerular diseases. For example, the presence of the hyalinosis lesion is not at all specific for the identification of diabetic nephropathy. This lesion may be seen in focal segmental glomerular sclerosis, in chronic pyelonephritis, and in other forms of glomerulonephritis as well. The diffuse lesion, particularly in its early stage of evolution, may also be confused with mesangioproliferative glomerular diseases. However, the lack or slight degree of hypercellularity in diabetic nephropathy may aid in its distinction from these forms. The addition of immunofluorescence and electron microscopic findings to the presence of the diffuse lesion may secure the diagnosis. Thus, the observation of linear staining of capillary walls for IgG and albumin by immunofluorescence, complemented by the occurrence of thickening of GBM and the absence of immune deposits by electron microscopy, eliminates the possibility of immune complex–mediated glomerulonephritis. Although the presence of the thick GBM should always suggest the possibility of diabetic nephropathy, such thickening may also be seen with hypertension or aging. If severe hyaline arteriolosclerosis is also present, then these findings are virtually specific for diabetic glomerulosclerosis even in the absence of the nodular lesion.

The nodular form of diabetic glomerulosclerosis is a distinct entity that should not be confused with other conditions. Several diseases involving the glomeruli may potentially confuse the unwary. The more common entities are amyloidosis, immunoglobulin deposition disease, immunotactoid and/or fibrillary glomerulonephritis, and membranoproliferative glomerulonephritis. Certain differences in staining patterns (Table 21.2), conformation of the nodules, and cellularity as well as immunofluorescence and electron microscopy allow the differentiation of these lesions from one another. Each is discussed in turn. Three rarer conditions, idiopathic nodular glomerulosclerosis (ING) (590), fibronectin glomerulopathy (591), and collagen glomerulopathy (592), are also shown in Table 21.2. The first will be discussed below. The second and third are discussed in Chapters 13 and 27, respectively, and are not considered further here. An algorithmic approach to diagnosis of diseases with organized deposits, which frequently show glomerular nodules, is provided by Herrera and Turbat-Herrera (172). These diseases may also be superimposed on diabetic nephropathy.

TABLE 21.2 Special stains in nodular glomerular lesions

Lesions	PAS	PAS/Jones	Masson	Congo red
Immunotactoid	+++	Neg	Blue	Neg
Diabetes mellitus	+++	Black	Blue	Neg
Light/heavy chain	++	Neg	Blue	Neg
Amyloid	Neg	Neg	Blue	+++
Idiopathic nodular	++	Black	Blue	Neg
Fibronectin	++	Neg	Red	Neg
Collagen	Neg	Neg	Blue	Neg

PAS, periodic acid-Schiff; Neg, negative.

Amyloidosis

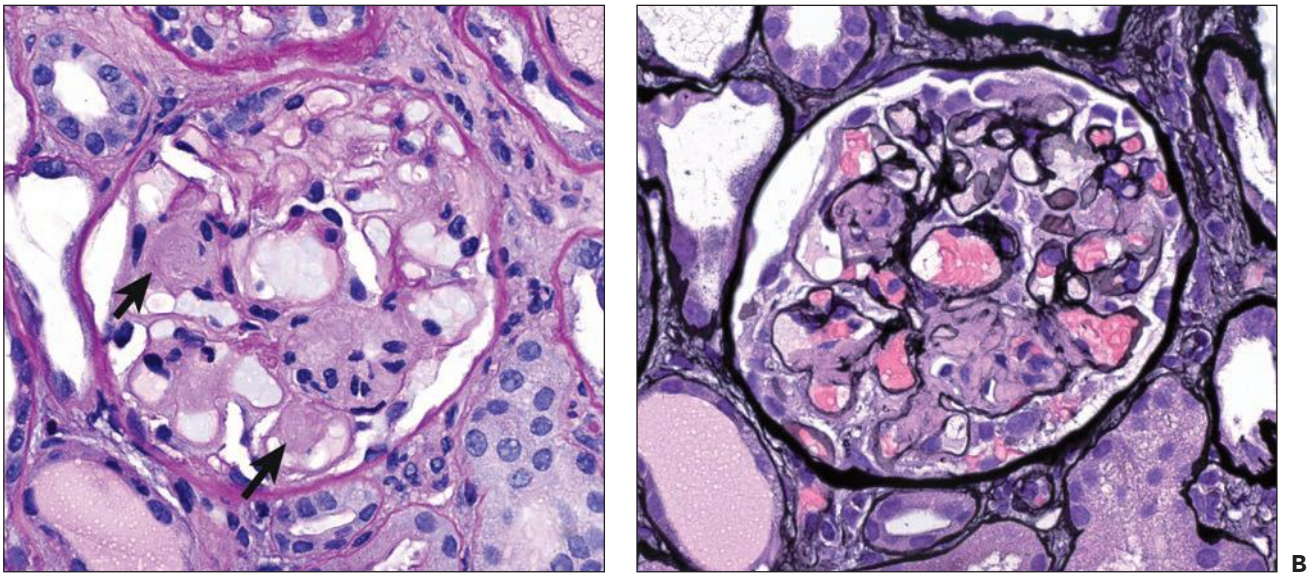
Amyloidosis is the easiest of the three diseases to separate from diabetic glomerulosclerosis. Although similar to the nodules of diabetes in distribution and irregularity of size, the mesangial nodules in this condition are virtually acellular. Furthermore, they stain with Congo red and appear apple green under examination with polarized light. In addition, amyloid tends to stain poorly with PAS and not at all with silver stains, unlike the nodules of diabetes (see Table 21.2) (Fig. 21.30). Capillary loops show variability in thickness unlike the uniform thickening seen in diabetic nephropathy. Electron microscopy demonstrates the classic appearance of rigid nonbranching fibrils measuring 8 to 10 nm in diameter. This lesion is further described in Chapter 22.

Immunoglobulin Deposition

Investigators now recognize that certain patients who excrete light or heavy chains of immunoglobulins in the urine, often in association with a plasma cell dyscrasia, may deposit these light chains in the kidney not as fibrils with the configuration of β-pleated sheets as in amyloid but as granular deposits (593). Because these do not stain with Congo red, differentiation from diabetic glomerulosclerosis is more difficult. This difficulty is verified by the fact that the condition was not identified until the late 1970s (594). Before that time, most cases of this condition were diagnosed as either diabetic glomerulosclerosis (in the absence of diabetes) or an anomalous form of membranoproliferative glomerulonephritis. The renal lesion in immunoglobulin deposition disease is characterized by increased lobulation of the glomeruli with nodules forming in the mesangium. Cellular proliferation often results in a picture resembling membranoproliferative glomerulonephritis, but in some glomeruli, the nodules may predominate (Fig. 21.31). Furthermore, the light or heavy chains frequently accumulate in the tubular basement membranes and produce thickening of these structures and a picture similar to diabetic glomerulosclerosis. These nodules and the materials around tubular basement membranes do not stain with silver (see Table 21.2). This condition is defined by immunofluorescence studies using kappa and lambda light chains or the four subclasses of IgG (595). Electron microscopy shows the accumulation of dense material on the endothelial aspect of the GBM and the outer aspect of the tubular basement membrane. This lesion is more fully described in Chapter 22.

Immunotactoid and/or Fibrillary Glomerulonephritis

Although these lesions may show nodules, they more frequently simulate the diffuse mesangial lesion of diabetes. However, early in these diseases, capillary loops tend to be variably thickened, rather than showing the uniform alteration typical of diabetes. The material stains brightly with PAS but does not take up the silver stain (see Table 21.2) (Fig. 21.32). Electron microscopy provides the definitive diagnosis with the characteristic fibrils or microtubules measuring between 15 and 60 nm in diameter. These lesions are more fully discussed in Chapter 23.



A **B**

FIGURE 21.30 Glomerulus from a patient with renal amyloidosis. Note lack of staining of nodules with both PAS *(arrows)* **(A)** and periodic acid-methenamine silver **(B)**. (**A**, ×440; **B**, ×400.)

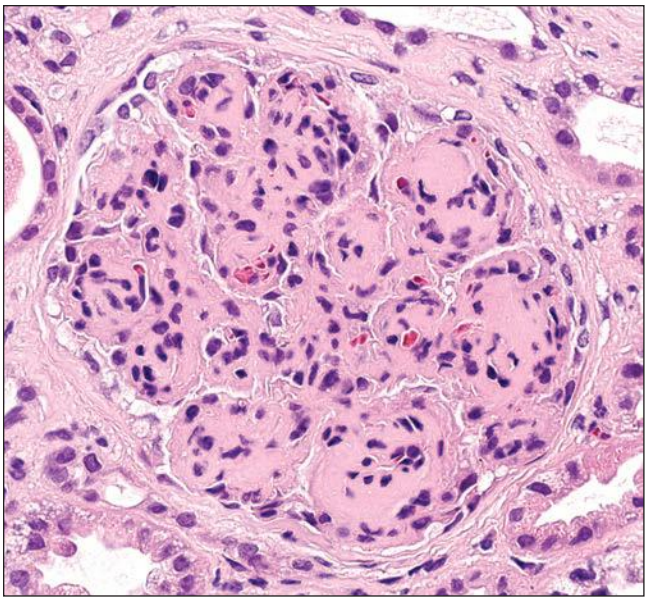


FIGURE 21.31 Glomerulus from a patient with κ light chain deposition. Mesangial increase and nodules are seen in both glomeruli accompanied by cellular proliferation. (H&E; ×420.)

Membranoproliferative Glomerulonephritis and Other Diseases With This Pattern of Injury

This pattern of glomerular injury may also be confused with diabetic glomerulosclerosis, but several differences aid in the distinction. First, membranoproliferative pattern of injury affects all glomeruli to a similar degree, in contrast to the nodular change of diabetic glomerulosclerosis, which affects only some of the glomeruli. Second, the change of membranoproliferative pattern of injury is uniform with respect to size of nodules and usually is manifest in all or most of the lobules of a given glomerulus. In contrast, the diabetic nodules vary in size and in number of lobules bearing them in any particular glomerulus. Third, increased cellularity is the typical finding in the mesangium of membranoproliferative pattern of injury, whereas in diabetes, one often sees only a mild increase in the number of cells, which are characteristically situated at the periphery of the nodule. Silver stains may show a double contour in membranoproliferative pattern of injury reflecting mesangial cell interposition with the production of a GBM-like material, producing the "double contour." The typical immunofluorescence finding of a peripheral lobular pattern of staining is suggestive of membranoproliferative pattern of injury. Electron microscopy demonstrates the presence of subendothelial and mesangial immune deposits in type I membranoproliferative glomerulonephritis and C3 glomerulopathies (596). Dense intramembranous ribbon-like deposits in the GBM, Bowman capsule, and tubular basement membranes are seen in dense deposit disease. These findings are more fully described in Chapters 8 and 9.

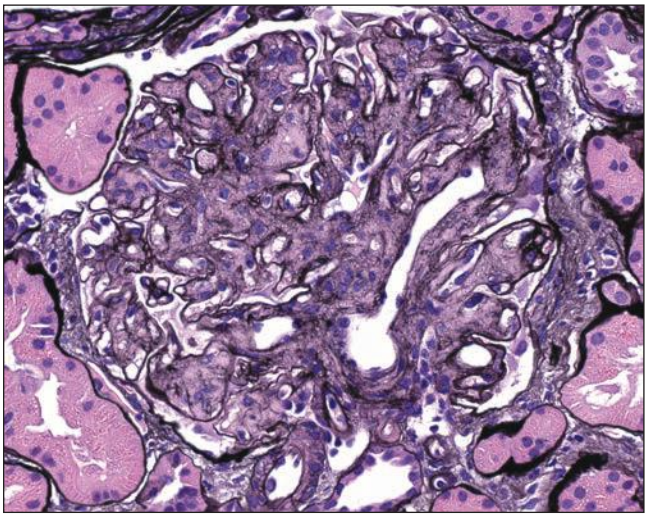


FIGURE 21.32 Glomerulus from a patient with immunotactoid (fibrillary) glomerulopathy with poorly formed silver-negative nodules and irregular thickening of the GBM. (Periodic acid-methenamine silver, ×260.)

Idiopathic Nodular Glomerulosclerosis

ING was first recognized as a unique entity by Alpers and Biava (597). Additional cases have been described (598–600). In all cases, the diagnosis of diabetic nephropathy must be excluded on clinical criteria. Markowitz et al. (590) had the largest series describing 23 cases allowing recognition of common clinical and pathologic features. The patients were most frequently older white men who presented with renal insufficiency and nephrotic range proteinuria. Additional common clinical features included hypertension, history of smoking, hypercholesterolemia, and vascular disease outside the kidneys. The pathology shows many of the characteristic findings of diabetic nephropathy including diffuse mesangial expansion with nodules, microaneurysms, capsular drops, and hyaline arteriolosclerosis. An unusual finding was the presence of endothelial-lined vascular spaces within the nodules confirmed by staining for CD34, an endothelial marker (Fig. 21.33) (590). Increased vessels were also noted in diabetics although to a lesser extent than in ING. Furthermore, the vessels in diabetics were at the periphery of nodules, while those in ING were more centrally placed in nodules (590). Nasr and D'Agati (601) proposed that smoking induced nodular glomerulosclerosis by the formation of advanced glycosylation end products, induction of oxidative stress, angiogenesis, and altered intrarenal hemodynamics. Li and Verani (602) examined 15 patients with ING and confirmed the high prevalence of smoking and hypertension. However, their patients also had increased incidence of obesity (60%) and overweight (27%) suggesting that these may be additional risk factors for this condition.

In summary, the well-formed nodular lesion with hyaline arteriolosclerosis is virtually pathognomonic of diabetes. However, the pathologist must exclude renal amyloidosis, immunoglobulin deposition disease, immunotactoid and/or fibrillary glomerulonephritis, membranoproliferative glomerulonephritis, and idiopathic nodular glomerulopathy. In the absence of the nodular lesion, the presence of diffuse mesangial sclerosis, typical linear immunofluorescence staining of glomerular capillary walls and tubular basement membranes for IgG and albumin, and thickening of the GBM by electron microscopy provide a constellation of findings typical of diabetic glomerulosclerosis.

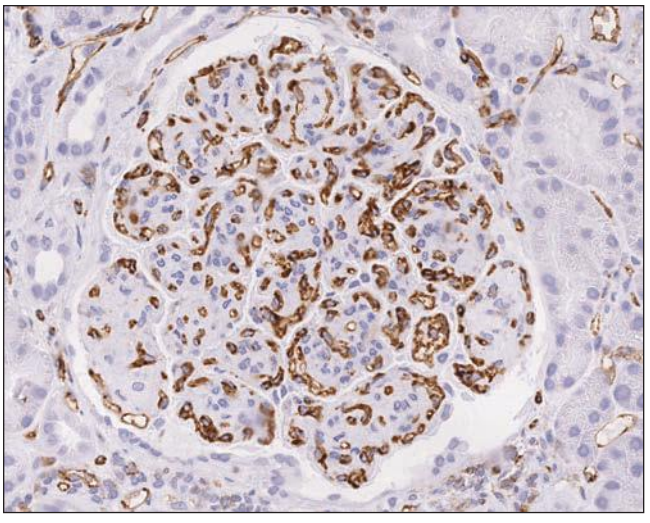


FIGURE 21.33 Glomerulus from a patient with idiopathic nodular glomerulosclerosis. Note numerous capillaries forming within centers of nodules as well as at periphery of nodules. (Immunoperoxidase stain for CD34, ×420.)

RENAL LESIONS OTHER THAN GLOMERULOSCLEROSIS

Patients with diabetes may develop nondiabetic renal diseases independent of or superimposed on diabetic nephropathy. As discussed above, this situation occurs more often in type 2 diabetes. Higher reported frequencies are seen in biopsy studies because unusual clinical features suggestive of nondiabetic renal disease often prompt a biopsy (189). Unbiased studies with proteinuria as the only criterion for biopsy show a frequency of 9% for nondiabetic renal disease (603). In an autopsy study, Carpenter et al. (178) found that 7% of diabetic patients had nondiabetic renal disease. In type 2 diabetes, vascular disease especially renal artery stenosis may complicate the clinical picture. Most categories of renal disease may be seen, including glomerulonephritis, pyelonephritis, papillary necrosis, and vascular disease.

Glomerular Lesions

The reported frequency of nondiabetic glomerular lesions varies from 5% to 71% in different reports depending on the criteria used to determine the necessity of biopsy (7,21,182,193,603–605). The occurrence of such lesions should be suspected in patients with rapid deterioration of renal function, persistent hematuria, or sudden onset of nephrotic syndrome especially before 8 years' duration of diabetes or in the absence of retinopathy (7,21,603–606). Tone et al. (606) assessed the specificity and sensitivity of duration of diabetes, retinopathy, microscopic hematuria, and granular casts to predict the presence of nondiabetic renal disease. They found that absence of retinopathy had the highest sensitivity (87%) and specificity (93%) followed by diabetes duration of less than 5 years with sensitivity of 75% and specificity of 70%. The remaining parameters were not as useful. Many other glomerular lesions have been described in diabetic patients. These include membranous glomerulonephritis, acute postinfectious glomerulonephritis, IgA nephropathy, crescentic glomerulonephritis, focal proliferative glomerulonephritis, membranoproliferative glomerulonephritis, lupus nephritis, nonspecific immune complex disease, and amyloid (21,182,189,603,605,606). These lesions may be present independent of diabetic glomerulosclerosis or superimposed on it. In addition, both minimal change disease and focal segmental glomerular sclerosis have been reported (182,607,608), although the occurrence of these two entities can only be definitely established in the absence of diabetic nephropathy. Whether the incidence of any type of glomerular disease is increased in the diabetic population or whether the coexistence of diabetic glomerulosclerosis with other glomerular lesions is a chance occurrence is not known. No studies in human beings have addressed the issue of increased susceptibility of diabetic glomeruli to the formation or deposition of immune complexes.

Renal and Urinary Tract Infections

Whether diabetic patients have an increased occurrence of renal and urinary tract infections is debated. The original studies relied on less strict histologic criteria for the definition of chronic pyelonephritis, and these criteria led to an overestimation of its incidence in patients with DM. The incidence of acute infection at autopsy is increased in diabetic patients as compared with nondiabetic patients (609). However, acute renal infection may occur as a terminal event in the diabetic patient and may be related to decreased host resistance to bacterial and fungal infections (610). Several recent studies have found a slight increase in incidence of urinary tract infections in patients with type 2 diabetes especially in older females as compared to controls (611,612). Scholes et al. (613) in a multivariable study found that diabetes was one of several parameters that did increase risk for acute pyelonephritis with an odds ratio of 4.1 (CI, 1.6 to 10.9). On the other hand, complications of severe urinary tract infection such as renal and perirenal abscesses, xanthogranulomatous pyelonephritis, and emphysematous pyelonephritis, a form of renal infection characterized by the presence of gas pockets within the renal parenchyma accompanied by extensive necrosis and abscess formation, do occur more frequently in diabetic patients (614–616). Emphysematous pyelonephritis occurs almost exclusively in diabetic patients. The pathologic features are described in Chapter 24. Patients present with the usual clinical symptoms and signs of urinary tract infection including fever, abdominal or flank pain, and pyuria (614,617), although evidence of severe sepsis is sometimes seen (615,617). Sixty-five percent of patients may also have increase in BUN and serum creatinine levels (614,617). A high index of clinical suspicion coupled with appropriate radiologic studies to confirm the diagnosis is crucial because emergency nephrectomy is the treatment of choice (615,617). Culture of surgical specimens has isolated *Escherichia coli* in 60% to 70% of cases and multiple organisms in 14% to 19% of cases (614,617). The formation of the gas and extensive necrosis are believed to result from multiple factors present in diabetic patients, namely, availability of glucose resulting in high levels of sugar fermentation, relative ischemia from vascular disease, and impaired host response to infection (614,616).

Papillary Necrosis

Papillary necrosis is increased in incidence at autopsy in diabetic patients (2.7% to 7.2%) compared with nondiabetic patients (0.6% to 1.4%) (618,619). Diabetes was present in 22% to

27% of cases in two large studies (618,620). The pathogenesis of papillary necrosis in patients with diabetes is due to several factors. First, relative ischemia is present because of vascular disease in diabetes. The medulla receives only 8% to 10% of the total renal blood flow, and a further reduction occurs at the tip of the papilla, so any additional decline may be expected to have adverse consequences (619). Second, infection is thought to play a role in the papillary necrosis that occurs in the setting of diabetes. Papillary necrosis occurs in one of every four patients with diabetes and acute pyelonephritis (621). One or the other of these factors may predominate in the individual patient (619). The clinician should also investigate the other known associations with papillary necrosis including analgesic abuse, sickling disorders, or urinary tract obstruction because these may also be present in diabetic patients.

Pathologic Findings

Gross Appearance

Both kidneys are affected and may be enlarged, normal, or reduced in size. The capsular surface may be granular in those cases with arteriolar narrowing. In those kidneys with acute infection, microabscesses may be observed on the subcapsular surface, sometimes with yellow lines extending through the cortex to the medulla. Varying numbers of papillae show yellow or grayish red, sharply defined areas (Fig. 21.34). The necrotic areas vary in shape and often have a congested border.

FIGURE 21.34 Severe papillary necrosis in a 64-year-old man who had diabetes and acute pyelonephritis as well as cancer of the prostate.

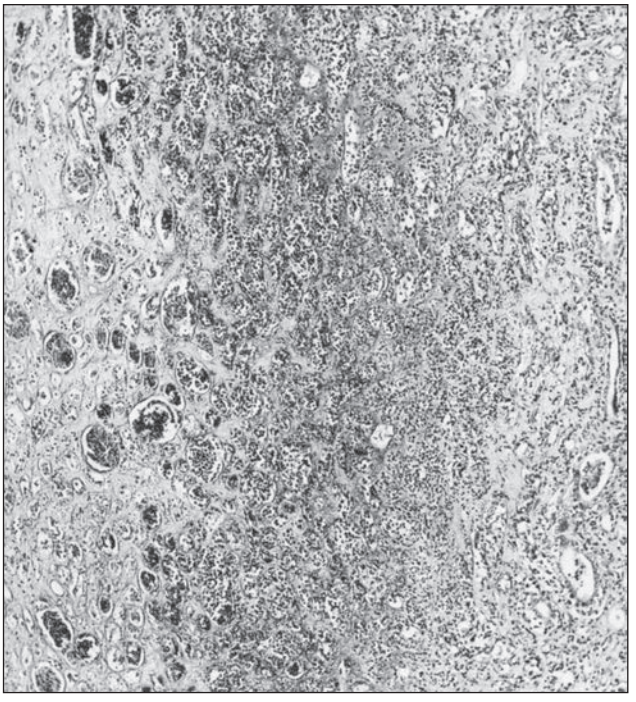


FIGURE 21.35 The zone of neutrophils at the junction of parenchyma with acute inflammation (*right*) and a necrotic papilla (*left*). (H&E, ×75.)

When the papilla has sloughed, one sees a ragged edge at the point of separation.

Microscopic Findings

Necrotic tissue is separated from living tissue by a dense zone of neutrophils (Fig. 21.35). In the necrotic area, tubular epithelium disappears, but powdered nuclear material and bacteria are often present. Inflammatory cells are not usually seen within the necrotic zone. The surface epithelium over the papilla is lost, particularly at the tip. In the nonnecrotic medulla adjacent to the neutrophil zone, one may see evidence of acute infection with neutrophils in tubules and interstitium. Interstitial sclerosis may be present when vascular insufficiency predominates over infection or when analgesic abuse is superimposed on the diabetes. The remaining kidney shows the changes of acute infection, chronic infection, or diabetes. The cortex may also show areas of tubular atrophy, interstitial fibrosis, and sclerotic glomeruli. Such zones result from ischemia, chronic infection, or obstructive nephropathy. The tubular systems draining into the necrotic areas are often dilated as in obstruction. In diabetes, papillary necrosis is most often a terminal event.

Clinical Aspects

Papillary necrosis occurs in both type 1 diabetes and type 2 diabetes. It has a female predominance (618,620). The most common risk factor is the presence of urinary tract infection. Microscopic hematuria and pyuria are more common in patients with papillary necrosis. In addition, other complications of diabetes such as retinopathy are frequent. The prevalence of papillary necrosis has shown a decrease. The appearance of papillary necrosis conveys reduced survival.

Vascular Changes

Arteriosclerosis and Arteriolosclerosis

Acceleration of arteriosclerosis is common in patients with DM occurring at an earlier age and with greater severity. Atypical angina and congestive heart failure are more common presenting features in diabetes (622). Female gender does not provide protection. Positive predictors of the risk of arteriosclerosis include age, hypertension, microalbuminuria, and high levels of cholesterol and serum sialic acid (623). The annual risk rate for coronary artery disease is 2% per year after 20-year duration of diabetes (568). Excess arteriosclerotic vascular disease occurs in patients without nephropathy if they have increased albuminuria (623). Nephrosclerosis may occur with or without diabetic nephropathy and may be worsened by hypertension. The appearances are described in Chapter 20.

Retinal Aneurysms and Other Microvascular Changes

Diabetic retinopathy remains the leading cause of blindness in the Western world (624). The elements of this retinopathy may be seen in other diseases, but the entire constellation, which includes thickening of basement membrane, microaneurysm formation, and increased permeability, is restricted to DM (625). The incidence of retinopathy generally increases with duration of disease. The progression of retinopathy is slowed by intensive metabolic control (626). The evolution of the pathologic changes leading to these appearances is well described (622,625,627). The earliest change in the retina of diabetic patients is the loss of capillary pericytes that help to control blood flow. Background retinopathy consists of microaneurysms, dot-blot hemorrhages, and hard exudates (627). The aneurysms lie on the venous side of the capillary network and occur in the capillaries that link the deep and superficial capillary plexus. The aneurysms appear as spherical or ovoid distensions, usually 20 to 30 μm in diameter (Fig. 21.36). Retinal microaneurysms are similar to glomerular capillary microaneurysms. The most severe manifestation of retinopathy is the proliferative form in which patients have extensive neovascularization of the retina, with inroad of vessels into the vitreous cavity and attendant bleeding and scarring leading to blindness. The prevalence of the proliferative retinopathy has not declined over the last 30 years (568).

Diabetic patients with advanced nephropathy usually have some form of retinopathy (628,629). Not all patients with retinopathy have renal disease, nor do all patients with glomerulosclerosis have retinopathy (568,605). Whether the pathogenesis of retinopathy and glomerulosclerosis is identical is not clear, although some of the factors overlap. A correlation exists between the degree of glucose control as determined by glycosylated hemoglobin and the occurrence of both renal and retinal complications (629). Klein et al. (629) also found a correlation between retinopathy and duration of diabetes. Severity of retinopathy correlated to severity of glomerular changes including GBM width, mesangial volume, GBM surface density, and a measure of glomerular injury (629). Recent studies have demonstrated that retinal vessel tortuosity, a change that precedes diabetic retinopathy, is an independent predictor of early diabetic nephropathy (630).

Blood vessels are affected in many different organs in diabetes. Microaneurysms have also been seen in the heart (631). Capillary basement membrane is also increased in thickness in other body sites (632,633). Steffes et al. (633) failed to find any correlation between thickness of capillary basement membrane in gastrocnemius muscle and severity of renal disease.

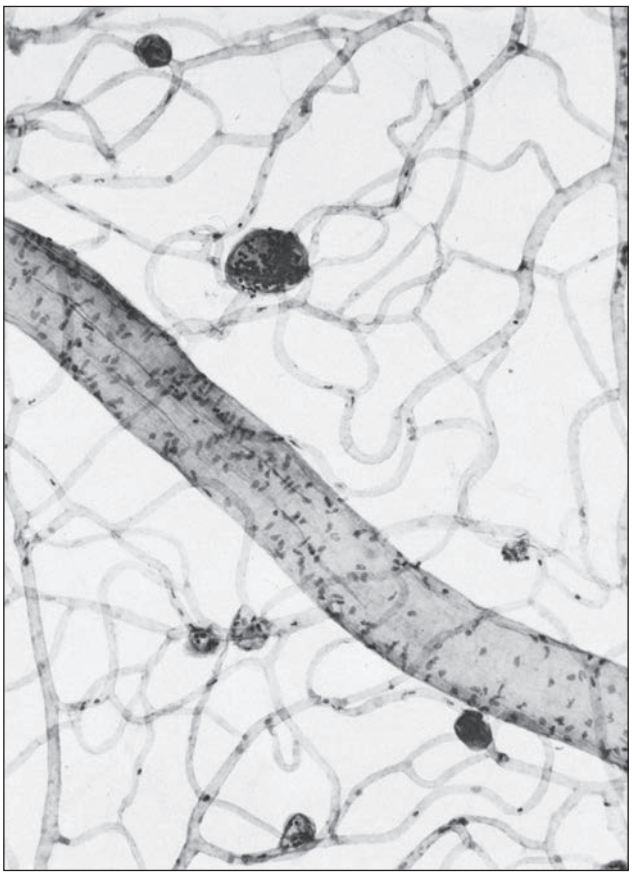


FIGURE 21.36 Retinal preparation to show microaneurysms. (×125.)

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CHAPTER 22

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Renal Diseases Associated With Plasma Cell Dyscrasias, Amyloidoses, and Waldenström Macroglobulinemia

HISTORICAL PERSPECTIVE

The important historical events more than 150 years ago that brought attention to an association between plasma cell dyscrasias and renal disease deserve recollection. Review of these

historical events allows us to follow chronologically how our understanding of the renal damage associated with dysproteinemias has advanced through the years.

Thomas Alexander McBean, a tradesman from London, sought medical attention in September 1844 because, while vaulting out of an underground cavern, he felt as if something had snapped within his chest, producing persistent intense pain (1). Dr. William MacIntire, McBean's attending physician, removed a pint of blood, applied a strengthening plaster to the chest, and recommended abstention from all bodily exertion, resulting in temporary relief and return to his "ordinary avocations." The improvement did not last long, and further treatment with steel and quinine was performed, with favorable results. However, in the following months, Mr. McBean eventually developed severe weakness, wasting, pallor, hepatic enlargement, pleuritic chest pain, and edema of the face and ankles. These new clinical developments forced a surgeon, whom he consulted, to "take blood from the arm to the amount of 1 pound and to apply leeches and blisters topically."

Dr. MacIntire observed peculiar abnormalities in his patient's urine, which was noted to be "opaque, acidic, and of high density with a specific gravity of 1.035" (1). Fifteen months after the initial incident, on October 30, 1845, Dr. Thomas Watson, a leading clinician in London at the time, evaluated Dr. MacIntire's patient and examined Mr. McBean's urine, corroborating the previous findings. Seeking help from a well-recognized chemical pathologist, Dr. Henry Bence Jones, was the logical way to proceed. The letter that Dr. Watson sent to Dr. Bence Jones remains an exact description of the urinary abnormalities that are encountered in many patients with renal disease and dysproteinemias. This was the beginning of a saga that deciphered the relationship between a totally unknown blood disorder and the kidney. Dr. Watson stated in this letter:

> The tube contains urine of very high specific gravity. When boiled it becomes slightly opaque. On the addition of nitric acid, it effervesces, assumes a reddish hue, and becomes quite clear; but as soon as it cools assumes the consistency and appearance which you see. Heat reliquifies it. What is it? (2)

Dr. Bence Jones took special interest in this specimen, analyzed the urine, and reported his findings. He deduced that the substance responsible for the urine abnormalities was not albumin because it was soluble in acid, and after performing a number of tests, he concluded that it was of a proteinaceous nature and referred to it as an "oxide of albumin, the hydrated deutoxide" (3). He calculated that the patient excreted 67 g/d of this substance. Today, we know this material as Bence Jones (BJ) protein, in recognition of his contribution to our understanding of its nature, in spite of the fact that it was really MacIntire who first discovered the abnormalities in McBean's urine. Bence Jones was the first to provide a detailed account of McBean's illness, referring to it as "… a hitherto undescribed disease, essentially malignant in nature … (affecting the) osseous system"; indeed, this is an accurate characterization of a previously unknown disease (3).

Mr. McBean's condition did not improve. He continued to have excruciating bone pain and developed intractable diarrhea, progressive generalized weakness, and emaciation. He died January 1, 1846, at 46 years of age.

An autopsy performed on Mr. McBean by Alexander Shaw revealed soft, friable ribs, sternum, and vertebrae, and they contained a "gelatiniform substance of blood red color and unctuous feel." The ribs were "brittle, soft, and easily cut with a knife," and as described by Dr. MacIntire, they "crumbled under the heel of the scalpel" (4). A diagnosis of *mollities et fragilitas ossium*, also known at the time as *mollities ossium*, quite descriptive terms for the disease in question (1,4–6), was made. Microscopic sections of the bones were examined by Dr. John Dalrymple, a surgeon at the Royal Ophthalmic Hospital in Moorfields, England, who documented the presence of abnormal cells in detailed drawings he made to illustrate his findings. These cells showed characteristics typical of malignant plasma cells (5), but plasma cells had not even been described at the time. Both Dalrymple and MacIntire believed that the disorder responsible for McBean's death was essentially a malignant disease of the bone. On his death certificate, the cause of death was "atrophy from albuminuria," (4) once again alluding to the renal component of this disorder as an essential manifestation of the disease process. The kidneys at autopsy were essentially normal on gross examination. It would take many years of clinicopathologic analysis and research to comprehend the scope of this patient's disease and to explain the different clinical manifestations.

Although the term *multiple myeloma* was introduced by von Rustizky in 1873 (7), the disease was rarely recognized until 1889, when Kahler published a case report (8). Kahler recognized that his patient had a similar substance in the urine to that described in McBean's urine. In 1900, Wright determined that multiple myeloma was a disease of plasma cells (9) when he recognized the similarity of the malignant cellular proliferation in this disease to cells initially described in 1875 by Waldeyer and fully characterized by Ramón y Cajal 15 years later in syphilitic condylomata (10). The association between plasma cells, their secretory products, and nephrotoxicity was not recognized until more than 50 years after McBean's death, in 1899 (11). Dr. James Ewing, lecturing to medical students in 1932, summarized the available knowledge by stating, "A very peculiar protein (BJ protein), specific of the disease and supposed to be derived from the adsorption of bone" (12). A definitive relationship between BJ proteinuria and the abnormal proteins seen in the serum of patients with myeloma was not demonstrated until 1956 in a study performed by Korngold and Lipari (13). These investigators determined that there were two types of pathologic light chains: κ and λ. Edelman and Gally demonstrated in 1962 that the light chains from the serum and BJ proteins of a myeloma patient were the same (14).

CLARIFICATIONS IN TERMINOLOGY: MULTIPLE MYELOMA AND OTHER MANIFESTATIONS OF DYSPROTEINEMIAS (PLASMA CELL DYSCRASIAS)

There are three clinical entities related to a diagnosis of dysproteinemia: multiple myeloma (often referred to as myeloma), plasma cell dyscrasia (dysproteinemia), and monoclonal gammopathy of unknown significance (MGUS). It should be noted that *plasma cell dyscrasia* and *dysproteinemia* are frequently used as generic terms for all of these disorders. Criteria for differentiating these three conditions have been clearly delineated by Durie (15). An understanding of the kinetics associated with plasma cell disorders is important for management and treatment of these patients (16,17).

Myeloma represents the most striking and advanced manifestation of a plasma cell dyscrasia. It is typically associated with lytic (punched out) bone lesions, which are often multiple. There is a monoclonal spike in the serum and/or BJ proteinuria resulting from the production by neoplastic plasma cells of either complete immunoglobulins or fragments of immunoglobulins. Finally, a significant increase in the number of bone marrow plasma cells (usually in the 15% to 20% range), often arranged in sheets with atypical cellular forms, is present. Criteria for clinical diagnosis of myeloma have been proposed (18,19) and approved by the American Society of Hematology.

Myeloma accounts for approximately 1% of all malignancies and 10% of all hematologic neoplasms (20). It is the second most common hematologic malignancy in the United States, with approximately 40,000 individuals suffering from myeloma at any time, and approximately 16,000 new cases are diagnosed every year in the United States (21). At any one time, there are approximately 250,000 patients with myeloma worldwide (21). The incidence of myeloma is approximately 4 in 100,000 individuals; it is higher among blacks than in the general population and more common in males than in females (22–24). The disease is most common with advancing age (mean age 65 years), but it is seen in individuals in the fourth and fifth decades of life. It is rare to find it in patients younger than 40 years of age (20), but there are reports of cases in the second decade of life. The incidence of this disease is rising as individuals live longer and survival is increasing. Renal insufficiency is a frequent complication of myeloma and the second most common cause of death after infection in these patients (20,23). Elevated serum creatinine was found in more than 50% of patients with myeloma, at initial examination, in a series of 869 cases described by Kyle (20). Approximately 15% to 20% develop acute renal failure, and a smaller percentage (about 10%) become dialysis dependent (25).

We use the terms *plasma cell dyscrasia* or *dysproteinemia* to denote a less than full-blown neoplastic plasma cell disorder. These terms are also often used as generic to refer to any lymphoproliferative or plasma cell disorder associated with production of an abnormal immunoglobulin or light chain. The affected patients often have circulating light chains in the serum or urine detected as a monoclonal (M) spike; they may have clinical manifestations, including renal findings, but the bone marrow is not diagnostic of myeloma. Although there may be a small increase in the number of plasma cells, they are not significantly atypical and are not clustered in sheets. In our experience, in approximately 5% of patients with dysproteinemia, the percentage of plasma cells is within the normal range (<5%). Lytic bone lesions are absent, and clinical manifestations are subtle or nondetectable. Routine bone marrow studies may be incorrectly considered as normal. When ancillary testing is performed (flow cytometry or immunomorphologic evaluations), a clone of plasma cells responsible for the production of the abnormal immunoglobulins is usually found (26). To establish monoclonality in these cases, immunophenotyping can also be performed on cytospin preparations using antibodies that recognize the major Vκ or Vλ subgroups or gene families and those that preferably identify free light chains (FLCs) (27).

The third group of patients with dysproteinemia have an isolated monoclonal M protein peak or gammopathy in the serum, and this condition is diagnosed as an MGUS. The amount of M protein must be lower than 3 g/dL, and there must be fewer than 5% plasma cells in the bone marrow (28–32). Other criteria include the absence or only small amounts of light chains in the urine, absence of lytic bone lesions, and no related anemia, hypercalcemia, or renal failure (28). The individual with an MGUS is otherwise normal. In patients with a diagnosis of MGUS, significant BJ proteinuria, even in the absence of recognizable renal disease, usually precedes clinical and laboratory manifestations of either myeloma or AL amyloidosis, but it may take more than 20 years for a clinical disease to develop (28,29). MGUS is found in approximately 3% of persons older than 70 years of age in Sweden (31). The prevalence of MGUS is higher in older patients, and only 4% of MGUS patients were younger than 40 years in a study by the Mayo Clinic group (28). Some of these patients essentially have "smoldering or indolent myeloma" and, with time, develop full-blown disease. In the Mayo Clinic study, 26% of the patients with MGUS developed multiple myeloma, Waldenström macroglobulinemia, or AL amyloidosis (28). Once a patient with a diagnosis of MGUS develops evidence of organ damage as a consequence of the circulating M protein, the diagnosis of MGUS is no longer tenable. A significant number of MGUS patients eventually develop renal disease. In fact, renal dysfunction is often the first systemic manifestation of progression.

The distinction between myeloma and MGUS based on bone marrow morphology is not reproducible, and it is virtually impossible to unequivocally separate one entity from the other (33). While the percentage of plasma cells is the most predictive feature of myeloma, the cytologic differences are not sharply defined. Interobserver variability is high in the assessment of morphologic atypia of plasma cells, and atypical plasma cells can be seen in patients with MGUS. This emphasizes the importance of identifying renal or other organ involvement in a given patient, because this finding objectively negates a diagnosis of MGUS. Recently, the term monoclonal gammopathy of renal significance has been used to refer to patients with renal manifestations associated with circulating monoclonal proteins and seemingly normal bone marrow evaluations (34).

The fundamental reason for making a distinction between plasma cell dyscrasia and multiple myeloma is because there is far greater consensus regarding the management of myeloma and renal disease compared to patients with renal disease who do not meet criteria for myeloma. The reality is that the pathogenesis for all these disorders is directly related to the overproduction of abnormal monoclonal light or heavy chains by a neoplastic plasma cell clone. The most recent literature stresses the indication for aggressive chemotherapy to eradicate the existing plasma cell clone. In fact, waiting to fulfill the criteria for myeloma before initiating treatment may deny the patient the early intervention that is needed to achieve optimum results.

Synthesis of Immunoglobulin Components by Plasma Cells and Abnormalities in Plasma Cell Dyscrasias

Plasma cells synthesize and secrete specific immunoglobulin molecules often with a minor excess of free κ or λ light chains. The plasma cells synthesize a variety of immunoglobulins, including IgG, IgM, IgD, IgE, and IgA, that can be detected

using serum protein electrophoresis (SPEP). Each immunoglobulin molecule is composed of two identical heavy chains (with molecular weight of approximately 50,000 Da each) and two light chains (molecular weight of approximately 25,000 Da each) linked by variable numbers of disulfide bonds. Both types of light chains consist of a common basic structure composed of a 107- to 111-residue amino-terminal variable (V_L) region and a 107-residue carboxyl terminal constant (C_L) domain. The V_L is the product of two genes, V (variable) and J (joining), that encode the first 95 to 99 amino acids and the remaining 12 amino acids, respectively. The light chain variable region of the germ-line DNA includes multiple V and J sequences. There are approximately 30 Vκ and Vλ germ-line genes that specify proteins on the basis of homology into Vκ 1, 2, 3, and 4 and Vλ 1, 2, 3, 6, and 8 subgroups (35–41). Variations in the V sequence result from the presence of approximately 30 Vκ and Vλ germ-line genes, with somatic mutations and differences resulting from recombinations of the V and J gene–encoded segments. These variations account for the variability in light chain pathogenicity and the site of pathologic action within the nephron. The carboxyl terminal of each light chain does not vary and is known as the constant C region. Each heavy chain has constant domains (C_H1, C_H2, and C_H3) and a variable domain (V_H). There are five types of heavy chains, namely, γ (IgG), α (IgA), μ (IgM), δ (IgD), and ε (IgE). Immunoglobulin G and IgA have variable numbers of disulfide bonds linking the heavy chains to each other and the heavy chains to the light chains. These characterize different isotypes of these Ig molecules, known as IgG1, IgG2, IgG3, and IgG4, as well as IgA1 and IgA2. Immunoglobulin A and IgG2 tend to exist in pairs of units known as "dimers" or may even polymerize to produce larger molecules. Immunoglobulin M exists primarily as a pentamer molecule composed of five Ig units.

Normal light chains synthesized by the plasma cells maintain a ratio of κ to λ of 2 to 1 in the serum. κ molecules occur predominantly as monomers or noncovalent dimers, with molecular weights of 22,000 and 44,000 Da, respectively, whereas λ molecules typically exist as covalent dimers. The V_H and V_L comprise the antigen-binding site. The C_H2 and C_H3 components are involved in effector functions such as binding to immune cells and host tissues and fixing complement. The synthesis of light chains occurs independently from heavy chains, and they combine in the rough endoplasmic reticulum to form the complete immunoglobulin molecule. The fact that light and heavy chains are synthesized independently is the pathogenetic basis for the existence of light chain– and heavy chain–related disorders as specific entities as well as occasional overlap entities (40,41).

In neoplastic plasma cell disorders, there is proliferation of a clone of plasma cells secreting a single type of Ig molecule or subunit that may be identified as a monoclonal peak on SPEP or on urine protein electrophoresis (UPEP) and characterized by immunoelectrophoresis or immunofixation (42). In some cases, only light chains are produced by the neoplastic plasma cells, and they are not generally detectable on SPEP but can be identified in the urine. The demonstration of a monoclonal protein in the serum or urine is important to corroborate a diagnosis of dysproteinemia. In a series of patients with myeloma reported by Kyle (20), a monoclonal protein was demonstrated in 90% of the patients using SPEP. The urine contains light chains in 60% to 80% of myeloma patients as detected by means of UPEP. Urinary light chains (BJ proteins) can also be found in the urine of patients with other B-cell neoplasms with plasmacytic differentiation. While FLCs readily circulate in the body, for heavy chains to be found in the circulation, they need to be released from the endoplasmic reticulum by binding with light chains. This is the reason why heavy chains do not circulate freely in normal individuals. Quantitation of serum FLCs (ratio of free kappa to lambda light chains) is very useful in the diagnosis and follow-up of patients with plasma cell dyscrasias/myeloma (25,43).

During the process of cellular replication and differentiation in the bone marrow, mutations typically take place when mature B lymphocytes are transforming into plasmablasts. The mutated plasmablasts produce a colony of identical mutated plasma cells or what is referred to as a *plasma cell clone* in a particular bone marrow site. The abnormal plasma cells eventually travel to additional bone marrow locations and other organs, disseminating the pathologic process and producing the various lesions seen in cases of advanced myeloma. Most malignant plasma cell disorders actively produce immunoglobulins, and these are generally composed of one type of light and one type of heavy chain.

In dysproteinemias, the normally controlled production of antibodies is replaced by an inappropriate production of larger amounts of immunoglobulin molecules by the bone marrow. The production of light and heavy chains may be unbalanced, resulting in free light or heavy chains. Imbalance of immunoglobulin production most commonly results in an excess of physicochemically abnormal light chains (39,43).

Furthermore, in dysproteinemic patients, biosynthesis of abnormal light chains, large, polymeric, or fragmented, has been documented in bone marrow cell cultures from patients with monoclonal immunoglobulin deposition diseases (MIDDs) and amyloidosis (40,41). It has become clear that mutations resulting in amino acid substitutions in the light or heavy chain molecules are crucial in determining their pathogenicity or absence thereof, along with the type of renal involvement. In some cases, certain physicochemical characteristics of these immunoglobulin components make them nephrotoxic, and even in cases where the production of these immunoglobulins by plasma cells is small, significant renal damage may occur.

Fewer than 1% of myelomas produce no immunoglobulin molecules (nonsecretory), and approximately 5% to 10% produce only light chains, which may only be detectable in the urine (44). The SPEP in these patients could be normal or shows nonspecific alterations.

The light chains in patients with plasma cell dyscrasia may be larger or smaller than normal, with molecular weights ranging from 12,000 up to 200,000 Da. Normal kappa light chains are monomeric and have a molecular weight of 25,000 Da, while lambda light chains tend to be dimeric with a molecular weight of 50,000 Da (25). Glycosylation of light chains contributes to an increase in their molecular weight. In a small number of myeloma cases (<5%), two different abnormal immunoglobulin molecules or fragments of these molecules are produced, indicating the presence of two distinct clones of neoplastic plasma cells (41). Immunoglobulin G is the most common immunoglobulin produced in myeloma cases (52%), followed by IgA (25%). Myelomas producing IgD, IgE, and IgM together account for fewer than 1% of all cases.

The primary structure of light and heavy chains is mostly responsible for whether a given molecule is pathogenic to the kidney or not, as has been clearly shown in studies with recombinant variable portions of light chains. Not all light chains from patients with plasma cell dyscrasias result in renal damage. Particular amino acid alterations will result in changes in the tertiary conformation of the proteins, leading to either partial or complete unfolding and changes in stability, potentiating aggregation (45–60). The three-dimensional configuration of a given light or heavy chain molecule can be predicted using computer modeling techniques; taking this information into account, the effects of a particular protein can be anticipated (50,52). It is known that λ light chains are preferentially associated with amyloidosis, while κ light chains are most common in light chain deposition disease (LCDD) (54,60–63). λ Proteins of two Vλ gene families, 6a and 3r, are typically associated with amyloidosis (54), and patients with λ 6 are the most common ones with renal amyloidosis. In LCDD, the majority of patients are κ Vκ 1 or 4 related. The first biochemical characterization of light chain deposits in tissue using extraction techniques was published by Picken et al. in 1989 (57). Changes in the stability and glycosylation of these light chains may also affect their ability to produce renal damage (66). Fanconi syndrome–related acute tubulopathy is almost invariably κ light chain related. In these patients, a specific amino acid substitution in position 30 of the variable portion of the light chain molecule accounts for the failure of complete processing and catabolism of the involved light chain in the proximal tubular lysosomal compartment (65,66). Biosynthetic data from studies of bone marrow plasma cells from patients with myeloma indicate that those light chains, which are heavier than normal, are usually glycosylated, and this alteration can make the light chains more nephrotoxic or change their pattern of nephrotoxicity (64).

Metabolism of Light and Heavy Chains in Normal Individuals and Pathologic Behavior in Patients With Plasma Cell Dyscrasia

Because light chains are low molecular weight proteins, they are freely filtered through the glomeruli and delivered to the proximal tubules. Glomerular clearance of light chains may be affected by a number of factors, including their physicochemical characteristics, size, isoelectric point, hydrophobicity, and state of aggregation. For example, light chain polymers and heavy chains do not cross the filtration barrier. Once the light chains are filtered by the glomerulus, 90% are reabsorbed by the proximal tubules, endocytosed, and catabolized through an endolysosomal process in the apical tubular regions, with their amino acids eventually returning to the circulation (67,68). This process is very efficient in normal individuals, with only a small amount of FLCs found in the urine. The cubilin-megalin receptor located on the brush border of proximal tubular cells working in tandem controls the endocytosis of the light chains (69–71). Light chain endocytosis occurs by a very specific, saturable, receptor-mediated process. There are a number of ligands that compete with light chains for brush border binding. The internalized light chains are then transported into vesicles, where the endosomal system catabolizes them. Hydrolytic enzymes present in the endosomes digest the light chains. Some of the process of light chain digestion appears to take place at the brush border itself, before endocytosis. The κ and λ light chain susceptibility to catabolism varies and accounts for the fact that the ratio of κ to λ light chains is reversed in the urine (2:1, λ to κ) (72).

In the setting of a plasma cell dyscrasia, the quantity of light chains in the filtrate may exceed the maximal reabsorptive capacity of the proximal tubular cells. When this occurs, the light chains pass into the distal nephron, where they may precipitate or remain in the tubular filtrate, resulting in light chain (BJ) proteinuria. Light chains precipitate out of solution when the urine is heated to approximately 56°C and redissolve as the temperature rises. As the urine is allowed to cool again, a precipitate forms, followed by dissolution as further cooling occurs. These are the characteristics documented by Dr. Bence Jones in Mr. McBean's urine (2). Normal patients may excrete small amounts of light chains (up to 50 mg/d), whereas in patients with myeloma, the light chain excretion may increase to 3 to 85 g/d (73,74). There is no apparent specific relationship between some of the characteristics of the monoclonal light chains excreted by individuals (i.e., chemical properties, subtype κ v λ, monomer vs. dimer, anionic vs. cationic) and the presence of pathologic findings demonstrated clinically or experimentally.

Normal light chains are not attracted to the mesangium and do not interact with mesangial cells. In contrast, some physicochemically abnormal light chains from patients with plasma cell dyscrasias interact with purported mesangial receptors and alter mesangial homeostasis.

Also, in patients with myeloma, the concentration of light chains reaching the kidneys is usually much higher, and the inability of the proximal tubules to properly catabolize the abnormal light chains leads to pathologic alterations. In these patients, the light chains commonly circulate as polymers that cannot be properly broken down by the endosomal/lysosomal system in the proximal tubules, enhancing their propensity to produce pathologic alterations. After the glomerular filtration barrier is compromised, as a result of monotypic light chains interacting with the glomerular basement membranes, such polymers may be freely filtered.

While there is a mechanism to deal with the small amounts of light chains that circulate in normal individuals, that is not the case concerning heavy chains, because they cannot be filtered though the glomerulus owing to their high molecular weight. If free heavy chains are released to the circulation, they interact with the capillary endothelium and mesangium, where they engage in pathologic processes. Presumably, the physicochemical characteristics of the particular heavy chains will dictate how they produce pathology. There is no information currently available on how heavy chains are processed by the kidneys, and the knowledge available regarding pathogenesis of heavy chain–related diseases is rather limited at this time. In Waldenström macroglobulinemia, the circulating IgM molecules become entrapped in subendothelial zones and generally do not significantly alter mesangial homeostasis nor produce tubular lesions.

LABORATORY DIAGNOSIS

The identification of a monoclonal protein in the serum and/or urine is important to confirm a diagnosis of dysproteinemia. Immunoelectrophoresis is routinely used to characterize the

monoclonal protein that is detected in serum or urine. SPEP is a good screening test for plasma cell dyscrasia, even though light chain secreting and nonsecreting plasma cell disorders lack a monoclonal spike. In these cases, examination of the urine for BJ proteins is important to make or solidify a diagnosis. The urine must be properly concentrated to detect small amounts of the monoclonal light chains. Immunoelectrophoresis or immunofixation may be necessary to confirm a diagnosis in some instances.

Immunofixation electrophoresis, a faster technique than immunoelectrophoresis, is the most sensitive and commonly used method available for the detection of monoclonal proteins. It is very helpful in identifying a monoclonal protein associated with a polyclonal increase of light chains, subtle bands associated with faint monoclonal or biclonal proteins, and monoclonal heavy chain fragments in the urine. Clarification of banding patterns noted on electrophoresis gels is possible by direct comparison of results. The superior resolution, simplicity, and enhanced sensitivity of immunofixation make it the diagnostic modality of choice to detect monoclonal gammopathies. One caution with immunofixation is that it requires precise dilution of the antibodies to avoid a prozone effect (42).

High-resolution electrophoresis (thin-layer agarose gels) may be combined with transfer onto nitrocellulose, followed by resolution of bands with monospecific enzyme-tagged antisera or monoclonal antibodies (Western blotting). This procedure is extremely sensitive, is more discriminating than immunofixation, and allows detection of minute amounts of monoclonal light chains (75). The technique can be utilized in selected instances when the monoclonal protein is in very small amounts.

Serum FLCs have become very important in the diagnostic algorithm clinically used to detect and follow plasma cell dyscrasias/myeloma. Serum concentrations of FLCs are dependent upon the balance between production of light chains by plasma cells and renal clearance. The normal serum FLC κ-to-λ ratio is 0.26 to 1.65. A plausible explanation for the inverted κ/λ ratio relates to the kinetics of FLC clearance with kappa molecules normally being monomeric and lambda LCs dimeric (25). If there is an increase of polyclonal plasma cells or renal function impairment, both κ and λ light chains will increase, but the ratio of κ and λ light chains will remain normal. In contrast, a monoclonal increase of either κ or λ light chains by a neoplastic clone of plasma cells will alter the ratio, providing a numerical indicator of clonality. Serum FLC immunoassays provide better sensitivity and precision than electrophoretic tests (25). They are particularly useful in the diagnosis and monitoring of patients with light chain cast nephropathy (myeloma kidney) (76). The high sensitivity of serum FLC immunoassays makes them also very useful in the initial screening for plasma cell dyscrasias.

RENAL INVOLVEMENT IN PLASMA CELL DYSCRASIAS

Renal involvement in dysproteinemia/plasma cell dyscrasias/myeloma is heterogeneous. Approximately 85% of all light chains with plasma cell dyscrasias are nephrotoxic. The morphologic manifestations vary, depending on the renal compartments targeted by the nephrotoxic light or heavy chains. In some instances, more than one renal compartment is affected, and combinations of different patterns of renal damage can be seen in the same patient. The majority of the nephrotoxic light chains (approximately 70%) affect the tubulointerstitial compartment and are referred to as *tubulopathic*. The other 30% of nephrotoxic light chains preferentially involve the glomerular compartment, producing glomerulopathies (*glomerulopathic* light chains). The physicochemical characteristics of the involved immunoglobulin molecule appear to be a crucial pathologic determinant. There are also some uncharacterized host factors that may influence the pathologic alterations and the degree of damage. Genetic polymorphism represents an important consideration that has not been studied. In this chapter, the light chain– and heavy chain–associated disorders will be discussed separately, but the reader must understand that on occasions they may be found acting in concert. Each of the diseases has specific clinical manifestations, pathologic findings, pathogenesis, prognosis, and management, and these specific features support viewing them as separate diseases. These diseases include the following:

- Light chain (myeloma) cast nephropathy
- Proximal tubulopathies, monoclonal light chain mediated
- Tubulointerstitial nephritis, monoclonal light chain mediated
- Deposition diseases including light chain (L), heavy chain (H), and light and heavy chain (LH) related (LCDD/HCDD/LHCDD)
- Amyloidoses including light chain (AL) and heavy chain (AH) related (AL/AH amyloidosis)

Light chain cast nephropathy, proximal tubulopathy, and tubulointerstitial nephritis are part of the spectrum of renal damage produced by tubulopathic light chains. The glomerular and vascular compartments are not typically affected by the tubulopathic light chains. Amyloidosis and the deposition diseases generally exhibit glomerular manifestations, but they are also commonly associated with tubulointerstitial and vascular pathology. In very rare circumstances, alterations in the vasculature (i.e., in AL amyloidosis) may be the predominant (77) or the first morphologic manifestation of renal involvement, preceding pathologic damage to other renal compartments. Combined patterns such as AL amyloidosis and LCDD, LCDD, and light chain cast nephropathy are uncommon (78–80) and may alter morphologic expressions of these disorders. For example, in 69% of these cases, in a series of 23 renal biopsies from patients with combined LCDD and light chain cast nephropathy, glomeruli do not display typical nodular glomerulosclerosis and appear essentially normal by light microscopy (81).

Infiltration of the renal parenchyma by neoplastic plasma cells is rare and usually occurs in terminal patients with myeloma (80). Renal insufficiency or failure because of renal parenchymal infiltration is very unusual. Neoplastic aggregates of plasma cells seen in the renal parenchyma (82) may be associated with malignant plasma cells in the urinary sediment (83).

Light Chain (Myeloma) Cast Nephropathy

Historical Perspective

Cast nephropathy was the first renal lesion to be recognized in patients with myeloma. It was well documented by Decastello in 1909 (84), but the first cases had been recorded

in the literature a few years earlier by Ellinger (11). In the early 1920s, Krauss (85) championed the concept of nephrotoxic light chains, but others noted that, at least in some patients, large amounts of BJ proteinuria were not always associated with renal insufficiency. Thannhauser and Krauss (86) hypothesized in 1920 that the tubular casts were concretions of serum proteins and BJ "albumose." In a comprehensive study by Bell in 1933 addressing renal lesions in myeloma, which included a complete review of the literature, he concluded that "it seems highly probable that casts are the chief cause of renal insufficiency resulting from multiple myeloma" (87). Other series of patients with overt myeloma have shown that light chain cast nephropathy is the most common lesion seen in these patients (88–90). This observation has not changed through the years (81,87,88,91).

Clinical Presentation and Laboratory Findings

The most typical presentation of cast nephropathy is acute renal functional deterioration or frank renal failure (91–93). It remains the most common cause of acute renal failure in patients with myeloma. In some cases, there are identifiable precipitating factors, such as dehydration, hypercalcemia, contrast media, nonsteroidal anti-inflammatory drugs, hyperuricemia, infections, nephrotoxins, or loop diuretics, such as furosemide. Renal biopsy may establish the diagnosis of underlying myeloma, or the patients may already have an established diagnosis of myeloma and are biopsied because of renal insufficiency to determine the renal lesion. After the diagnosis of light chain cast nephropathy, approximately 90% of patients are found to have overt myeloma (89). These patients frequently also have nephrotic range proteinuria, predominantly composed of light chains. Routine urinalysis using a dipstick, which primarily detects albuminuria, commonly fails to pick up light chain proteinuria.

Gross Pathology

There are no specific gross features in kidneys with light chain cast nephropathy (82,88,90). The kidneys may have subcapsular pathology, including granularity and occasional petechiae, but these are likely related to vascular disease (90). The mean weight of the kidneys from patients with myeloma cast nephropathy was 166 g in one autopsy series (90).

Light Microscopy

The glomerular and vascular compartments are normal in appearance or show changes related to other preexisting conditions, that is, benign nephrosclerosis. The most striking changes are in the tubulointerstitial compartment when tubular casts are present in the distal nephrons (Fig. 22.1) (91–93). In fact, most casts are located in the collecting ducts, and if the medulla is not included in the specimen, the diagnosis may be missed. The typical casts exhibit irregular, angulated, and geometric shapes; fracture planes; and occasionally a lamellated internal appearance, attesting to their protein-rich composition, which imparts to them a firm and often brittle consistency, as they interact with Tamm-Horsfall protein (94–96). In some casts, the fragments come together in a jigsaw puzzle–type of arrangement, which is quite peculiar and characteristic (Fig. 22.1A). Casts in the proximal tubules and even in the urinary space are sometimes seen as a result of retrograde filling. An interstitial inflammatory reaction, predominantly with mononuclear inflammatory cells and sometimes eosinophils, often accompanies the tubular casts. Tubulopathic light chains associated with nephron obstruction can elicit an interstitial inflammatory reaction by stimulating cytokines (94–99). The interstitial inflammatory process may be as important as the obstructive process (97).

The casts contain predominantly light chains, Tamm-Horsfall protein, and polymorphonuclear cells (99), but they may also include cell debris from tubular damage (94–96,98). The epithelial cells in the tubules with casts often appear reactive and, at times, enlarged. Multinucleated cells of purported macrophage origin may also be seen inside tubules surrounding the casts (Fig. 22.1B), and it has been postulated that these cells migrate from the interstitium through the tubular basement membranes into the tubules (100–103). It also has been proposed that these multinucleated giant cells derive from transdifferentiation of tubular cells to a histiocytic phenotype (104). If the casts break through the tubular basement membranes, then a multinucleated giant cell reaction may be elicited in the adjacent interstitium surrounding the expelled material. These giant cells have also been shown to exhibit a macrophage phenotype. Ultrastructurally, these casts may exhibit fibrils with ultrastructural features of amyloid (105). The casts are generally eosinophilic and generally weakly

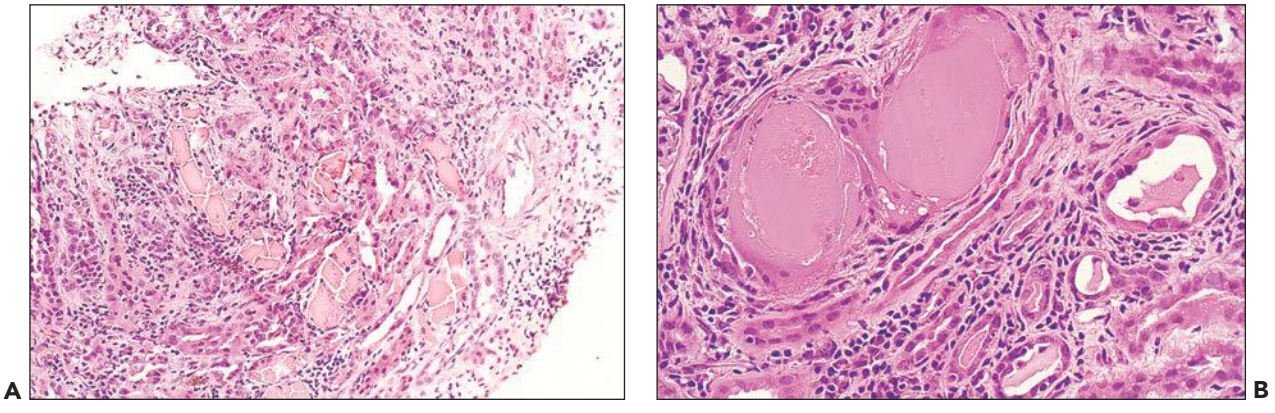

FIGURE 22.1 Light chain cast nephropathy. A: Typical distal nephron casts with brittle consistency, resulting in fracture planes. (H&E, ×350.) **B:** Multinucleated cell reaction around cast. (H&E, ×500.)

periodic acid-Schiff (PAS)-positive; however, there is significant variability in the tinctorial characteristics, as the composition of these casts may be quite variable. In some instances, the tubular casts are PAS-negative, and this may be a helpful diagnostic clue. In rare cases, the casts are composed exclusively of, or contain, crystals (Fig. 22.2). Whereas the morphology of casts may be quite characteristic, there are cases in which the morphology is not pathognomonic. In some of these atypical cases, immunofluorescence may be helpful. However, some cases require careful clinicopathologic correlation for accurate interpretation. The extent of cast formation correlates with the degree of interstitial fibrosis, tubular atrophy, and dropout, and there is also a correlation with renal function in many but not all cases (106).

Interestingly, some of the casts are congophilic and, upon polarization, elicit apple green birefringence; they also exhibit thioflavin T and S positivity (105). Certain histochemical and staining properties of renal tubular casts in human multiple myeloma and in "mouse myeloma" are similar to those of amyloid (107).

Immunofluorescence

The glomeruli and vasculature reveal no specific findings. Tamm-Horsfall protein can be demonstrated in the casts, because they form as a result of interactions between this protein and light chains (94–98). Albumin can also be found in the casts. Monotypic (restricted) light chain staining (either κ or λ) of the casts is only seen when the casts have been formed acutely and not when they have remained in place for a prolonged period of time. In a significant number of the cases, there is trapping of the other light chain, and as a consequence, fluorescence staining of similar intensity is noted for both light chains (108). When there is fluorescence for both light chains, the light chain involved in the plasma cell dyscrasia usually predominates, but the degree of one light chain predominating over the other is quite variable and, in some cases, it is difficult to unequivocally determine that there is definitive monoclonality. Silva et al. (103) found that the tubular casts contained the light chain identified in the urine in more than 50% of 40 patients with multiple myeloma.

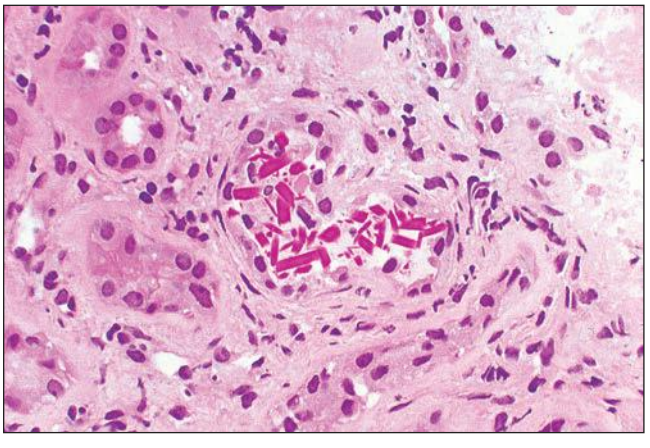

FIGURE 22.2 Light chain cast nephropathy. Distal nephron cast containing crystals. (H&E, ×500.)

Electron Microscopy

The glomeruli and the vasculature are unremarkable. In most cases, the casts contain abundant fibrillary material admixed with cellular debris. Granular, electron-dense material is seen in many casts, and their specific light chain identity can be substantiated by employing ultrastructural labeling techniques (108–110).

In selected cases, the casts are composed of variably sized and shaped crystalline structures. Such casts are fairly specific for light chain cast nephropathy (108–110), and the diagnosis can be confirmed by using ultrastructural immunogold labeling to demonstrate monoclonality when immunofluorescence studies fail to make the diagnosis (108–110). Immunogold labeling is a far more sensitive and specific technique than immunofluorescence; however, it is not available in most laboratories.

Etiology and Pathogenesis

Casts, in general, form in the distal nephron, and light chain casts are not an exception (85). Local factors combine to optimize cast formation. At this site, Tamm-Horsfall protein, produced by the thick ascending limb of the loop of Henle, is most abundant and provides a perfect nidus for cast formation. The casts form as a result of coaggregation of Tamm-Horsfall protein and light chains (94). The light chains are delivered to the distal portion of the nephron when they exceed the proximal tubule threshold for light chain reabsorption and/or after damage to the proximal tubules impairs reabsorption (95).

It was proposed that a high isoelectric point in the monoclonal light chain predisposed to cast formation (111–116), but this theory has not found universal acceptance. Another determinant of cast formation is pH (117). It has been shown that cast-forming monoclonal light chains bind to a common portion of the peptide backbone of Tamm-Horsfall protein, with the carbohydrate moiety in this protein being responsible for facilitating coaggregation. The binding site for Tamm-Horsfall protein on monoclonal light chains is located within the CDR-3 (complementarity-determining region 3) (118–120). The secondary structure and key amino acid residues on the CDR-3 of FLCs are important determinants of the molecular interaction with Tamm-Horsfall protein (121). These findings lend support to the current view that the structure of the pathogenic light chain must be such that certain interactions occur. The slower fluid flow in the distal nephron is a contributing factor to effective cast formation.

In 1976, Koss et al. produced an obstructive renal lesion in mice by the intraperitoneal injection of a light chain from a patient with light chain cast nephropathy (122), and Solomon et al. published similar findings in 1991 (111). Clyne et al. proposed in the late 1970s that electrostatic interactions between various proteins involved resulted in precipitation and cast formation (112). Microperfusion of rat tubules with light chains purified from the urine of patients with light chain cast nephropathy has reproduced the distal nephron obstructive lesion in the research laboratory, further attesting to the importance of the physicochemical characteristics of a given light chain in the pathogenesis of the distal nephron lesion (119,120,123,124).

Myeloma casts have been found to be resistant to urinary and macrophage metalloproteinases, making their elimination difficult in some cases (125). The destructive interstitial nephritis that accompanies this lesion has been attributed to

rupture of the basement membranes of tubules with spillage of the cast contents, including Tamm-Horsfall protein, into the interstitium, leading to the release of potent cytokines and other mediators and resulting in potential irreversible interstitial damage (97,126,127), but experimental evidence indicates that direct tubular damage can also activate cytokines leading to recruitment of inflammatory cells (126,128).

Differential Diagnosis

The light microscopic appearance of the tubular casts in light chain cast nephropathy is frequently pathognomonic, and there are no other conditions that show similar findings (108). Crystals in casts should create a strong suspicion for a diagnosis of light chain cast nephropathy (108,110). However, when the casts are not classic in appearance, the nephropathologist must carefully evaluate all immunomorphologic data available to make a final determination. When monoclonality cannot be demonstrated by immunofluorescence, the diagnosis of cast nephropathy may be suspected but not confirmed. The clinician must then conduct the necessary studies to confirm the suspicion or rule out this possibility. The differential diagnosis should include nephropathies with cast formation and acute tubulointerstitial nephritis, especially when associated rapid deterioration of renal function. In rare circumstances, light chain protein excretion is increased in unrelated conditions (129). In rifampin-associated light chain proteinuria, a pathologic picture similar to that of myeloma cast nephropathy may occur (130). A similar morphologic picture that has been seen in some patients taking antirejection drugs such as tacrolimus and rapamycin used in combination in cases with delayed graft function can produce intratubular cast formation indistinguishable morphologically by light microscopy from light chain cast nephropathy. However, these casts do not contain monoclonal light chains. It has been suggested that rapamycin-induced toxic tubular damage represents an important mechanism in the pathogenesis of this lesion (131).

Treatment, Course of the Disease Process, and Prognosis

The great majority of patients with light chain cast nephropathy have a clearly identifiable plasma cell dyscrasia and meet the criteria for myeloma (approximately 90% of these patients) (89). This is responsible for the alternative diagnosis for this condition: myeloma cast nephropathy. The main therapy of myeloma cast nephropathy is aimed at avoiding the formation of additional casts by reducing the amount of circulating light chains, which is most efficaciously accomplished by treating the plasma cell dyscrasia and facilitating the clearance of existing casts (132). Plasmapheresis, especially in younger patients, has been used to acutely decrease the concentration of circulating light chains, while the chemotherapy decreases plasma cell mass and diminishes light chain secretion (133). This therapeutic strategy is particularly useful in patients with acute renal failure. To facilitate the clearance of existing tubular casts, proper hydration is of utmost importance. Maintenance of a high urine output (aiming at about 3 L/d) is the goal (134). Loop diuretics must not be used, and other agents that promote cast formation or produce renal damage should be avoided. These include radiocontrast agents, nonsteroidal anti-inflammatory drugs, and any nephrotoxic agents. Infections, hypercalcemia, and electrolyte imbalances should be promptly treated. Alkalinization of the urine may facilitate solubility of BJ proteins, but by itself it is of virtually no value.

Colchicine was promoted as an agent that would prevent the formation of new casts as a consequence of its effect on Tamm-Horsfall protein removing its carbohydrate component (94–96,135), but the clinical benefit of colchicine treatment remains doubtful. Cysteamine, a reducing agent, may be used to aid in dissolving the existing casts, and vincristine can disrupt casts already in place. Dimethyl sulfoxide has also been used to dissolve casts (136).

While renal function is compromised, many of these patients require dialysis (137,138). Dialysis is also recommended for those with acute-onset renal failure (135,136,138). There is also a role for plasmapheresis to decrease circulating nephrotoxic light chains (97). In addition, recent experimental data in an animal model support a role for small molecule inhibitors of the interaction of Tamm-Horsfall protein and light chains for treating light chain cast nephropathy (121).

Aggressive chemotherapy should be considered seriously and administered to all newly and previously diagnosed patients who are in otherwise relatively good health (132). Alkylating agents and prednisone not only directly act on the proliferating plasma cells but also result in a decrease of proteinuria and directly impact favorably on renal function (132,134–136). The treatment of those patients who do not meet minimal criteria for a diagnosis of myeloma is more controversial. Melphalan and prednisone have been used with good initial results in 50% to 60% of these patients (132,136). However, the trend is to be even more aggressive with patients with low tumor burden to effect a cure of the underlying plasma cell dyscrasia.

Dialysis should be instituted early to avoid uremia compounding the usual complications of the disease itself. About 20% of these patients die within the 1st month (138). Renal function improves in about 54% of the patients who present in acute renal failure when the plasma light chain concentration is decreased (132). However, progressive loss of renal function occurs in the majority with time, especially if the myeloma cannot be adequately controlled. The prognosis has not changed significantly in the last 30 years.

The overall median survival for patients with myeloma cast nephropathy and renal failure has been reported to range from 13 months (137,139) to 20 to 30 months, with a 5-year survival rate of 18% to 54% (20,138,139). Renal transplantation is generally not considered a viable therapeutic avenue because of the high risk of recurrence.

Proximal Tubulopathies, Monoclonal Light Chain Mediated (Proximal Light Chain Tubulopathies)

Historical Perspective

Most of the initial publications linking renal damage to myeloma concentrated on cast nephropathy. However, early reports indicated that direct tubular damage by nephrotoxic light chains was an important pathologic mechanism. In 1921, Löhlein first reported crystalline inclusions in proximal tubules in a patient with multiple myeloma (140). The inclusions were also seen in Fanconi syndrome associated with proximal tubulopathy in patients with plasma cell dyscrasias (141). In 1963, Costanza and Smoller (142) and, in 1975, Maldonado et al. (143) suggested that proximal tubular damage was an important pathogenetic mechanism in a subset of patients with myeloma and

renal damage. Clyne et al. (144) injected BJ proteins intraperitoneally in rats and produced intracytoplasmic inclusions in the proximal tubules. However, the glomerular filtration rate did not decline, leading the authors to conclude that although tubular alterations could occur, there was no direct association with renal failure. When these experiments were repeated 5 years later using intravenous infusion of BJ proteins, the same investigators demonstrated that severe reduction in glomerular filtration rate occurred, but in these animals, a significant component of distal tubule cast formation was also noted (112). DeFronzo et al. (145) indicated that the degree of renal failure correlated best with tubular atrophy rather than obstruction and that some patients developed defects in urine-concentrating ability and acidification. Ultimately, however, damage to proximal tubules by some nephrotoxic light chains was clearly demonstrated in experimental nephron microperfusion studies by Smolens et al. (120) and Sanders et al. (123). Some light chains were capable of producing both distal nephron obstruction and proximal tubule damage (123,124). In a clinical study, renal biopsies from patients with myeloma were found to have evidence of proximal tubular damage (146). Pote et al. (126) have further emphasized the role of nephrotoxic light chains in proximal tubular damage and demonstrated experimentally the direct toxic effect of some tubulopathic light chains on proximal tubules. Subsequently, the morphologic spectrum of proximal tubulopathy was demonstrated to include lesions with and without crystalline inclusions, various lysosomal alterations (including "indigestion/constipation"), acute tubular necrosis, and an association with interstitial inflammatory response in some patterns (147–150). Thus, more recently, the term "light chain proximal tubulopathy" has been proposed to encompass all of the above entities (147,147a,148).

Clinical Presentation and Laboratory Findings

It is not difficult to conceptualize proximal tubular damage in patients with plasma cell dyscrasias, because light chains are usually metabolized in proximal tubules. The delivery of excessive amounts of physiochemically abnormal light chains to the proximal tubules may lead to overload of the lysosomal system, followed by release of lysosomal enzymes and tubular cell damage. This type of tubular damage may be seen in combination with other renal manifestations in patients with plasma cell dyscrasias, and the clinical manifestations that predominate in those cases may be those related to the other conditions (i.e., those associated with AL amyloidosis or LCDD). When this lesion is found by itself, the clinical presentation may be variable and range from rapidly progressive renal failure, or acute renal failure, to a slowly progressive increase in serum creatinine (76,108,110,142,145–150). Some patients with this pattern of renal damage present with proximal tubular dysfunction, including aminoaciduria, phosphaturia, and glucosuria. Other clinical manifestations include subnephrotic range proteinuria, uricosuria, and at times, renal tubular acidosis type II (of proximal tubular origin) (141,142). This is the typical constellation of findings in acquired Fanconi syndrome. Among proximal tubulopathies associated with crystalline inclusions, virtually all cases that have been described have been κ light chain–related, indicating that the composition of the light chains is a crucial determinant of this specific pathologic manifestation (147–149). In contrast, proximal tubulopathies not associated with crystals are commonly λ light chain–associated (147–150). An abnormal clone of plasma cells is detected in approximately 50% of these patients at first presentation (151), and there may be a prolonged interval between the diagnosis of Fanconi syndrome and clinical evidence of myeloma. In one case, the renal abnormalities preceded the demonstration of an underlying plasma cell disorder by 16 years (143).

Similar proximal tubular dysfunction is noted in those cases where the lysosomes present in proximal tubules are filled with monotypic light chains and are unable to release their hydrolytic enzymes (termed "lysosomal indigestion with constipation syndrome") (147,148,151).

While some degree of clinically insignificant proximal tubular damage may be present in most patients with plasma cell dyscrasias and nephrotoxicity (146), the proximal tubulopathies can be responsible for rapidly or slowly progressive renal failure (110,148). Tubular damage associated with light chain–related Fanconi syndrome occurs in fewer than 5% of patients with renal involvement in plasma cell dyscrasias (93,110).

Gross Pathology

Macroscopic features of kidneys from patients with Fanconi syndrome associated with proximal tubular damage have been documented only rarely. The kidneys have been noted to be enlarged with pale cortical areas (141), findings similar to those seen in association with acute tubular necrosis, regardless of the etiology.

Light Microscopy

Proximal tubular damage by nephrotoxic light chains may lead to crystal formation (tubulopathy with crystalline inclusions) or noncrystalline accumulation of monoclonal light chains in the proximal tubules with variable degrees of tubular damage and lysosomal alterations (108,109,147–150). In cases with crystal formation, which are frequently associated with clinical Fanconi syndrome, needle-like intracytoplasmic tubular inclusions may be identified with PAS and trichrome stains (146–150,152–155). Crystalline inclusions may also be seen in the neoplastic plasma cells in the bone marrow (140) and in other cell types (152–155). There are a few reported cases with Fanconi syndrome containing typical intracytoplasmic crystals in tubular cells coexisting with myeloma cast nephropathy (156). In noncrystalline proximal tubulopathy, three distinct patterns of proximal tubular injury have recently been described: (a) tubular damage with features of acute tubular necrosis, (b) basolateral deposition of the light chain with interstitial inflammatory response, and (c) lysosomal accumulation with enlargement and atypical lysosomal forms (lysosomal indigestion/constipation pattern) (147,147a).

The early changes include vacuolization of the tubular cells followed by apical blebbing with loss of surface microvillous borders, desquamation, and fragmentation (Fig. 22.3A). In some cases, tubular integrity is compromised to the point where only tubular outlines remain (*arrows* in Fig. 22.3B). Evidence of tubular regeneration with mitotic figures can be seen.

Immunofluorescence

Monoclonal light chains may be detected in the cytoplasm of the tubular cells corresponding to the localization of the light chain in lysosomes (147,147a,148). In other cases, even though staining for both light chains is noted, there is obvious predominance of the pathogenic light chain (110,157,158). This is explained by filtration of the nonpertinent light chain and uptake by the

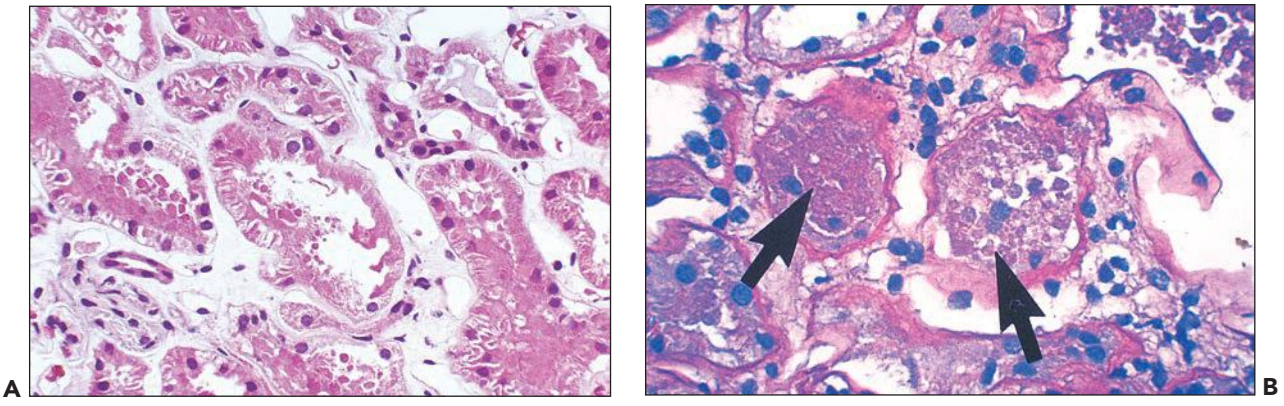

FIGURE 22.3 Proximal tubulopathy, monoclonal light chain related. A: Early mild changes in the proximal tubules, with fragmentation and desquamation of tubular cells. (H&E, ×500.) **B:** Severe tubulopathy with loss of tubular integrity resulting from cell necrosis (*arrows*) with loss of nuclei. (H&E, ×750.)

proximal tubules. The preferential or monotypic staining for a type of light chain should be taken as a clue that the tubulopathy is related to an underlying plasma cell dyscrasia. However, the absence of detectable staining for either of the light chains does not rule out this condition. Sometimes, the abnormal light chains, partially digested in the lysosomes, are not detected by the available antisera. In the Fanconi syndrome–associated cases, the needle-shaped proximal tubular inclusions may fluoresce intensely for κ (extremely rarely for λ) light chains and be very easy to detect (64,65,158). However, in the majority of the cases, fluorescence evaluation does not aid in identifying the cytoplasmic inclusions. Pronase digestion of paraffin-embedded tissues may be of value in detecting the monotypic staining (159).

Electron Microscopy

Proximal tubular damage can be confirmed ultrastructurally. In experimental work and in clinical material, lysosomal proliferation, tubular cell vacuolization and fragmentation, apical cytoplasmic blebs, and segmental loss of microvillous borders are generally present, although in some with variable degrees of severity. The lysosomal system appears overactive, and large and atypical lysosomes are often found (Fig. 22.4) (108–110,148,150,160,161). One subset of these patients exhibit proximal tubules packed with large, atypical lysosomes that obscure other organelles (147). In cases associated with Fanconi syndrome, there are needle-shaped, round, or rectangular to rod-like, electron-dense structures in the cytoplasm of the proximal tubular cells. The needle-like inclusion bodies can appear crystalline (Fig. 22.5) or fibrillary (147,147a,162). At high magnification, the crystalline inclusions sometimes exhibit parallel linear arrays. These structures, as well as the large, atypical lysosomes, can also be labeled for the specific light chain using ultrastructural immunogold techniques (Fig. 22.5) (108–110,148,158,161).

Etiology and Pathogenesis

The pathogenesis of this type of renal damage is directly related to the inability of the lysosomal system to degrade the nephrotoxic light chains, resulting in overload ("clogging") of the lysosomes in the proximal tubules with or without crystal formation (123,147,148,160,161). When immunogold labeling is performed, the lysosomes are found to be overfilled with the monotypic light chain that they are unable to properly degrade. In most cases, lysosomal overload causes release of their proteolytic enzymes into the cytosol, leading to cytoplasmic vacuolization, simplification, and even frank necrosis (147). As a consequence, fragmentation, desquamation, and apical blebbing of the proximal tubular cells occur with accompanying segmental or total loss of microvillous borders. Light chains are a ligand for the megalin receptor (70). Silencing megalin and cubilin genes responsible for production of receptor proteins for light chain on the brush border of proximal tubule cells inhibits light-chain endocytosis and ameliorates toxicity (163). Most cases of Fanconi syndrome are associated with the κ1 subgroup, most originating from two germ lines: LCO2 and LCO12 (162). In the case of Fanconi syndrome–associated proximal tubular damage, the partially digested light chains form the fibrillary or crystalline inclusions in the cytoplasm of the proximal tubules. The crystalloid structures have been shown to contain an incomplete monoclonal κ light chain with a truncated NH-terminal fragment corresponding to the variable domain that is necessary for crystallization to occur (64,65,162). These partially digested fragments result from degradation by cathepsin B, and they do not bind Tamm-Horsfall protein except in very exceptional cases. This observation explains why cast nephropathy is so rarely associated with Fanconi syndrome. Elegant experimental work by Sirac et al. (162) has produced a transgenic model of this disorder that closely resembles its human counterpart.

Differential Diagnosis

In proximal tubulopathies without crystalline inclusions, the main differential diagnosis is acute tubular necrosis from other causes. The best way to make an unequivocal diagnosis of light chain–related acute tubulopathy with acute tubular necrosis is by demonstrating monoclonal light chains in association with the lesion in question using immunofluorescence, electron microscopy, immunoelectron microscopy, or a combination of these techniques (108,109,147,147a). Unfortunately, the commercially available antibodies to κ and λ light chains do not always detect the abnormal light chain deposited in the kidneys. Good clinicopathologic correlation may be helpful in solidifying this diagnosis. In the case of light chain–associated Fanconi syndrome, the presence of the characteristic tubular crystalline

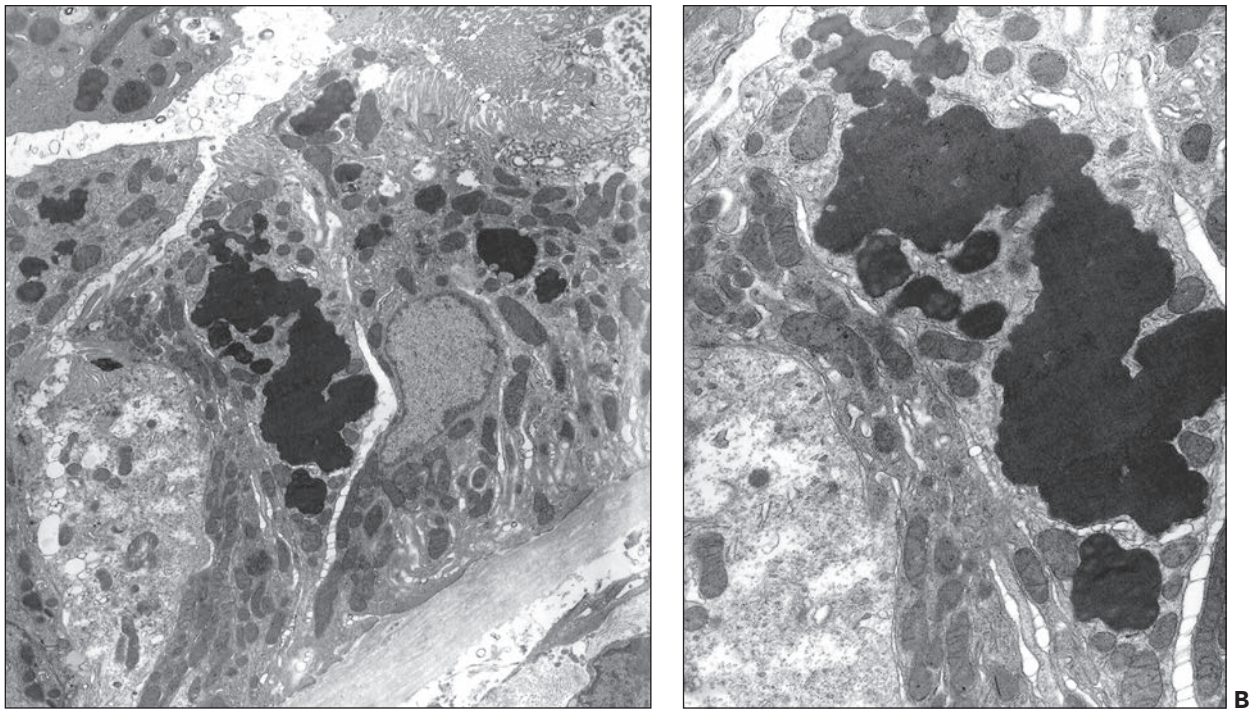

FIGURE 22.4 Proximal tubulopathy without crystalline inclusions, monoclonal light chain mediated. Atypical lysosomes in the proximal tubular cells exposed to tubulopathic light chains with segmental loss of the microvillous border. Transmission electron microscopy. (Uranyl acetate and lead citrate.) (**A:** ×11,000; **B:** ×17,500.)

cytoplasmic inclusions and the demonstration that these contain monotypic light chains suffice to make a solid diagnosis (147,148,154,155,157,158).

It is important to consider the possibility of tubular overload being responsible for the staining of light chains in proximal tubular cells. To confirm a diagnosis of light chain proximal tubulopathy, there must be monoclonality for the pertinent light chain and morphologic evidence of tubular damage. Moreover, these findings should be accompanied by clinical evidence of renal dysfunction in order for a diagnosis to be rendered with certainty.

Treatment, Course of the Disease Process, and Prognosis

This lesion is often seen in conjunction with other patterns of kidney injury, and the clinical course and prognosis of light chain–associated acute tubular necrosis or tubulopathy as a specific entity have not been critically analyzed. The disease

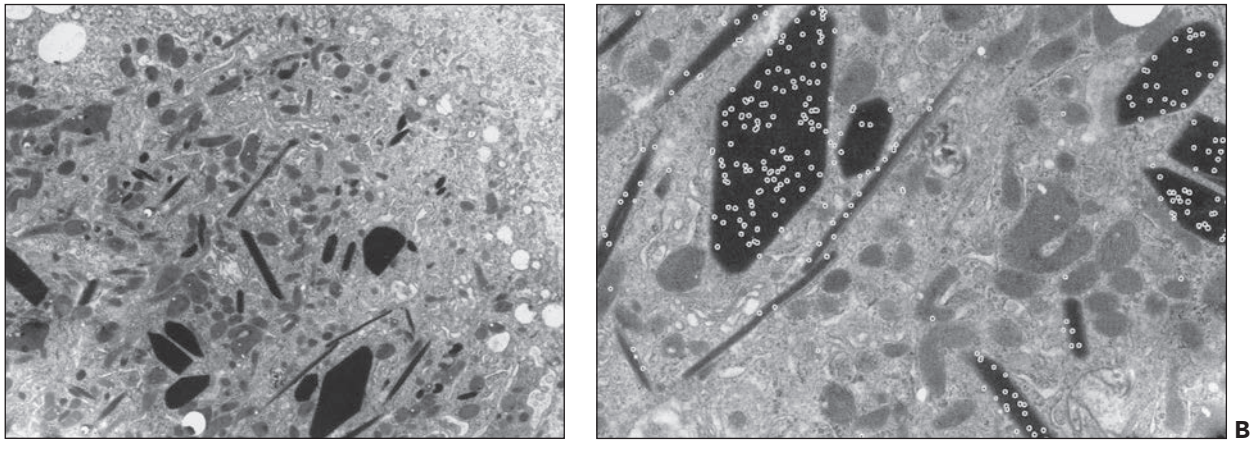

FIGURE 22.5 Acquired light chain–related Fanconi syndrome. A, B: Crystalline cytoplasmic inclusions labeled for κ light chains in the proximal tubular cells. **B:** Intense gold labeling for κ light chains in cytoplasmic inclusions. Ten-nanometer gold particles have been traced with computer-assisted technology to highlight the labeling. Transmission electron microscopy. (Uranyl acetate and lead citrate.) (**A:** ×8500; **B:** ×12,500.)

course and prognosis of the concomitant process tend to prevail in these combined patterns of disease. However, anecdotal cases indicate that, by itself, this lesion is fully reversible if the circulating light chains can be controlled (161). Therefore, aggressive treatment of the underlying plasma cell dyscrasia, together with clinical support while the tubules are regenerating, is the standard of care. Some patients may require temporary dialysis during the acute renal failure episode. It is also important to realize that when combined lesions are present (i.e., LCDD and light chain–associated acute tubular necrosis), the tubulopathy may be the main culprit responsible for the renal failure (110,159,160). If tubular function can be reinstated, renal failure can improve dramatically. This lesion can recur in transplants (148).

There are variable degrees of tubulopathy, and the mild forms may not be of significant clinical importance. The long-term effects of recurring acute tubular necrosis in the setting of an underlying plasma cell dyscrasia are not known. More careful clinicopathologic studies are needed to clarify the overall importance of this lesion on patients' prognosis and renal survival.

Because this may be the only renal pathology seen in a biopsy, a definitive diagnosis in a patient with a circulating paraprotein represents objective morphologic evidence of organ damage and should be taken as an indicator that treatment of the plasma cell dyscrasia is warranted.

Tubulointerstitial Nephritis, Monoclonal Light Chain Mediated

This is currently a frequent pattern of renal damage associated with plasma cell dyscrasias (147,147a,164). It mimics acute tubulointerstitial nephritis. It is important to recognize it so that its association with an undiagnosed underlying plasma cell dyscrasia by detecting monoclonal light chain deposition in association with the tubulointerstitial pathology can be established and to rule out other forms of tubulointerstitial nephritis.

Historical Perspective

Two patients were reported in the 1980s with inflammatory tubulointerstitial changes and/or isolated tubular basement membrane monotypic κ light chain deposits. The patients had myeloma with no associated glomerular or vascular light chain deposition, and they were considered atypical LCDD cases (40,165). For many years, it has been recognized that in patients with cast nephropathy, interstitial inflammation may be a significant finding. Some patients with known myeloma and renal insufficiency show no evidence of cast nephropathy or any other forms of plasma cell dyscrasia–associated pathology, but the biopsy shows a patchy (or diffuse) interstitial inflammatory infiltrate associated with tubulitis, providing a clue that such a lesion could be part of the spectrum of plasma cell–associated renal pathology. A series of eight such patients was compiled in 2006 (164) to bring attention to this pattern of light chain–related renal disease, which could be confused with an acute tubular interstitial nephritis unrelated to the plasma cell dyscrasia because of the similarity in histologic findings (166). All but one of these patients and the two previously published cases were κ light chain related, but this lesion may be seen in association with monoclonal λ light chains as well.

Clinical Presentation and Laboratory Findings

Among patients with plasma cell dyscrasias and related renal disease, this morphologic pattern accounts for at least 10% of cases (147,147a,164) and is increasingly recognized more often (147a). All the patients with this disease process have presented in acute renal failure, and the cause is either unknown or related to a plasma cell dyscrasia, most often overt myeloma. The patients are usually older than 50 years of age. Serum and urine electrophoresis have shown preferential association with κ light chains. Nonnephrotic range proteinuria may be found. Serum creatinine is quite variable, but it is generally more than 3 mg/dL at presentation (164).

Gross Pathology

The kidneys from two patients exhibiting this lesion in an autopsy series showed normal weight and no specific gross findings (82).

Light Microscopy

The glomerular and vascular compartments are unremarkable. The tubulointerstitial compartment shows an inflammatory process composed predominantly of lymphocytes and plasma cells but also containing variable numbers of eosinophils associated with tubulitis (Fig. 22.6). The inflammatory process can be subtle or intense, focal, or generalized. Tubular damage

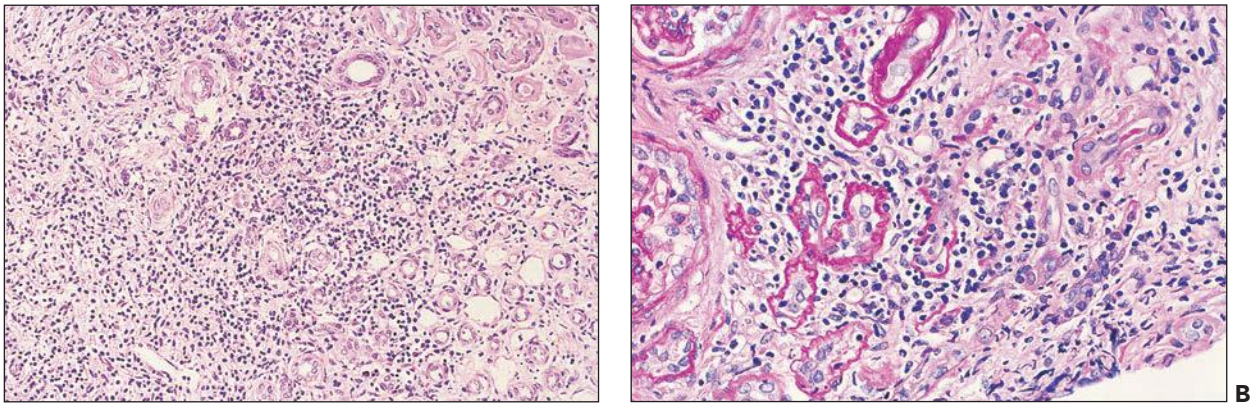

FIGURE 22.6 Tubular interstitial nephritis, monoclonal light chain mediated. A: Intense interstitial inflammation associated with lymphocytes extending through the tubular basement membranes into the tubules. (H&E, ×350.) **B:** This is better seen on PAS stain. (×500.)

can be variable but is frequently marked. There is no tubular cast formation. The absence of casts in multiple sections taken from autopsy kidneys represents substantial evidence that this lesion is clearly separable from light chain cast nephropathy and that it represents a specific pattern of renal damage in patients with plasma cell dyscrasias (164). Because the diagnosis requires the demonstration of monotypic light chains along the tubular basement membranes, it cannot be established by light microscopy alone.

Immunofluorescence

Linear monotypic light chain staining may be demonstrated outlining the tubular basement membranes in association with the most intense interstitial inflammation. In a small subset of patients, the staining may be focal. There may also be intracytoplasmic staining in proximal tubular cells for the monotypic light chain (147,164). As with acute tubulopathy, there are cases in which staining for both light chains is present, but if the pertinent light chain is more prominently stained, this finding supports the diagnosis.

Immunohistochemistry

In some of these cases, monotypic light chain staining deposition along tubular basement membranes in areas with interstitial inflammation and tubulitis can be clearly demonstrated using immunohistochemistry (164). In these cases, monotypic staining for the pertinent light chain can be striking, whereas there is no staining for the other light chain (Fig. 22.7).

Electron Microscopy

No specific glomerular or vascular changes are identified. Specifically, neither there are light chain deposits nor there is evidence of amyloid deposition. In some cases, there may be focal deposition of light chains, represented by punctate to powdery, electron-dense material along the outer aspect of the tubular basement membranes (147,147a,164). The lysosomal system may be prominent in the proximal tubules. By ultrastructural immunogold technique, distinct labeling for monotypic light chains can be demonstrated along the tubular basement membranes in areas with prominent interstitial inflammation and in tubules exhibiting tubulitis. Both proximal and distal tubules may be labeled, but the findings are usually more striking along distal tubules (108–110,164). Highly concentrated monotypic light chains are also noted in proteinaceous material in tubular lumina, which does not organize into well-formed casts (150).

Etiology and Pathogenesis

The inflammatory interstitial reaction that may occur in these cases is probably induced by the binding of pathogenic light chains to the tubular basement membranes, which alters intrinsic tissue antigens and promotes the release of cytokines, resulting in chemoattraction and activation of interstitial mononuclear inflammatory components (166,167). Light chains may reach the tubular basement membranes by transcytosis after reabsorption or by passive tissue diffusion from peritubular capillaries (164). Conceptually speaking, this pattern of renal disease could be considered as a type of LCDD with pathologic manifestations restricted to the tubulointerstitial compartment (165), but it indeed represents a peculiar, monoclonal light chain–related tubulointerstitial manifestation that warrants consideration here as a separate entity.

Differential Diagnosis

An important differential diagnosis is acute allergic tubulointerstitial nephritis (168–170). When eosinophils are present, a hypersensitivity reaction is an important consideration in the differential diagnosis. It is imperative for an accurate diagnosis to demonstrate that monotypic light chains are associated with the tubulointerstitial compartment using ancillary diagnostic techniques. Interestingly, proximal tubulopathy with basolateral monoclonal light chain deposition can also be associated with an interstitial inflammatory response (147,147a). There are no light microscopic findings that allow separation of the light chain–related type of inflammatory tubulointerstitial nephritis from the other types of acute tubulointerstitial nephritis.

Treatment, Course of the Disease Process, and Prognosis

This disease may require steroid therapy, just as needed for any type of tubulointerstitial nephritis (97) and treatment of the plasma cell clone producing the tubulopathic light chains. The use of steroids in this setting is still under clinical investigation,

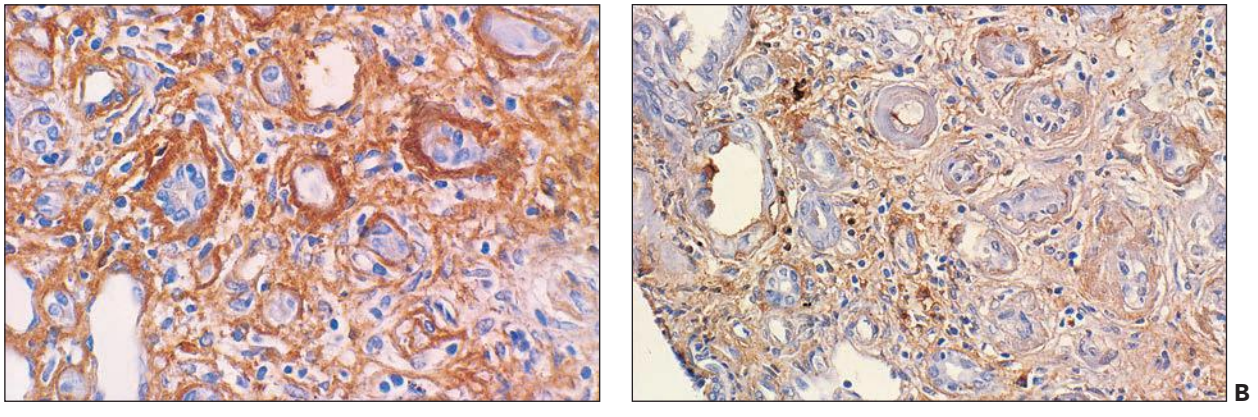

FIGURE 22.7 Tubular interstitial nephritis, monoclonal light chain mediated. Note labeling of tubular basement membranes for κ **(A)** but not λ **(B)** light chains in an area with prominent interstitial inflammatory activity. Immunohistochemistry for κ **(A)** and λ **(B)** light chains. (Peroxidase antiperoxidase stain, diaminobenzidine as marker, ×500.)

and no definitive results are available at the present time. There are no specific studies addressing kidney survival or the clinical course of this disease.

MONOCLONAL IMMUNOGLOBULIN DEPOSITION DISEASES

MIDDs are systemic disorders characterized by deposition of monoclonal immunoglobulins in many organs, but the kidneys are the most commonly involved. In most cases, light chains are the immunoglobulin components that deposit in tissues, giving rise to LCDD. More recently, heavy chain–associated monoclonal deposition disease, also referred to as *heavy chain deposition disease* (HCDD), has been recognized. Interestingly, the pathologic findings in both light and heavy chain deposition diseases (LHCDDs) are quite similar (89,108,156,171,172). Our understanding of the pathogenesis of light chain–related deposition diseases is more complete than that of heavy chain–related diseases. This is mainly because light chain–associated diseases were described long before their counterparts, and they are more prevalent. Cases of combined LHCDD account for about 10% of all cases (156). The morphologic spectrum of LCDD is extensive, so routine staining of renal biopsies for κ and λ light chains is imperative to identify unusual and early manifestations of these disorders.

Historical Perspective

In 1957, Kobernick and Whiteside showed nonamyloid glomerular abnormalities in patients with myeloma and recognized the similarity of these lesions with diabetic nephropathy (173). About 10 years later, Abrahams et al. (174) reported a myeloma patient with renal disease and deposition of subendothelial material in the glomeruli described as "coarse and more granular than the glomerular basement membrane." The next year, Rosen et al. (175) reported a similar case, with what he described as glomerular "osmiophilic subendothelial densities." It was Antonovych et al. (176) in 1974, who first recognized the association of the ultrastructural findings noted above with the deposition of κ light chains. Randall et al. (177) in 1976 published two autopsies from patients with plasma cell dyscrasias and documented the widespread pathologic findings of a disease that he referred to as "systemic LCDD." HCDD was first described 17 years later by Aucouturier et al. (178), who emphasized that in this disorder, instead of deposition of light chain immunoglobulin components, there were deposits composed of monotypic heavy chains. In 1985, Jacquot et al. (78) were the first to describe three cases of LCDD associated with light chain (AL) amyloidosis.

Light Chain Deposition Disease

Clinical Presentation and Laboratory Findings

There is no significant sex predilection for this disease, but males predominate slightly in most series (7:5). The average age of patients with LCDD with renal manifestations is 55 to 60 years. At the time of diagnosis, acute renal failure is present in 30%, and a similar percentage is dialysis dependent. Most (more than 90%) present with proteinuria, and the average protein excretion per 24 hours is in the nephrotic range (generally 4 to 5 g/d) in 53% of LCDD patients. Interestingly, full-blown nephrotic syndrome is noted in a minority of these cases (156). Seventy-eight percent of LCDD patients present with hypertension, 52% have hematuria, and varying degrees of renal insufficiency are detected in 95% of these patients (155). In selected LCDD cases, tubular dysfunction is the predominant clinical manifestation (179,180). As the clinicopathologic heterogenicity of this disease is better appreciated, patients are diagnosed earlier, and the manifestations of renal insufficiency tend to be less pronounced (179–184).

Rapid deterioration of renal function occurs as the disease advances, if not treated aggressively. Renal biopsy preceded any other clinical signs or evidence of dysproteinemia in 70% of the cases with pure LCDD (156,172). Classic features of underlying myeloma are present in more than half of these patients, but a significant number of patients with LCDD have either a normal bone marrow biopsy and aspirate or rather unimpressive plasmacytosis at presentation (108,110). Careful evaluation of the plasma cells in the latter case using either immunohistochemical techniques or flow cytometry can identify a monoclonal population of plasma cells, albeit a small clone in many cases. Biosynthetic studies of the plasma cells often demonstrate paraprotein production, even when serum and urine electrophoresis fail to show any abnormalities (40). In autopsy of patients who died with a clinical diagnosis of myeloma, approximately 3% to 5% exhibited LCDD (78,84). This figure is conservative, as the less characteristic cases of LCDD are not diagnosed because immunofluorescence and electron microscopy of the kidneys were not part of the evaluation of these autopsies. The most commonly involved light chains in these cases are κ, and subtypes IV and I predominate (185). The ratio of κ to λ is 9:1 in LCDD (156).

Bone marrow biopsy and aspirate revealed sufficient criteria to diagnose myeloma in 35% of LCDD cases, osteolytic lesions in 13%, and hypogammaglobulinemia in 33%. Of the patients with LCDD at the time of clinical presentation and diagnosis, 39% carried a diagnosis of MGUS (186). Up to 30% of patients with MIDD have no detectable monoclonal proteins in urine or serum. Even with use of the most sensitive techniques available, the percentage of patients falling into this category remained at 15% to 20% (186).

When LCDD coexisted with light chain cast nephropathy, 82% of these patients presented with acute renal failure, and 64% required dialysis (55,89). Renal biopsy preceded any clinical evidence of dysproteinemia in 64% of the patients with combined LCDD and cast nephropathy. Cases of combined cast nephropathy and LCDD are mainly associated with κ light chains. LCDD can also occur in combination with light chain–associated (AL) amyloidosis (75–77) and HCDD, in which case it is called LHCDD (156). The paraprotein composition of LHCDD is typically IgG-κ or IgG-λ (156).

Gross Pathology

There are only two autopsy studies in patients with myeloma performed after LCDD was described in 1976. Three patients with LCDD were noted in the series by Ivanyi et al. (88,187), and the mean weight of the kidneys was 271 g. Herrera et al. (82) reported the autopsy findings in 77 patients with myeloma. These cases comprised approximately 10% of all cases with myeloma that died during the period of time this study covered at the institution. There were two cases with LCDD, both with small kidneys with average weight of 130 g and with evidence of surface scarring attributed to coexistent vascular disease.

Light Microscopy

The most characteristic finding in patients with LCDD is nodular glomerulopathy (Fig. 22.8) that mimics the pattern of nodular glomerulosclerosis in diabetic nephropathy (108,188–195). Capsular drop and hyaline cap lesions are not present in LCDD, and the absence of these features helps in the differential diagnosis from diabetic nephropathy. In addition, the mesangial nodules in LCDD are more evenly distributed, although there is significant variation depending on the stage of the disease process (60,61,184,189,190,194,195), whereas the nodules in diabetic nephropathy tend to be asymmetric. The mesangial nodules are argyrophilic and composed of extracellular matrix proteins admixed with monotypic light chains (196,197), and the principal matrix protein deposited is tenascin (197). In silver methenamine-stained sections, there may be lamellation of the peripheral portions of the mesangial nodules. Mesangial hypercellularity accompanies the increase in extracellular matrix in some of the cases. The peripheral capillary walls are variably thickened, and the capillary wall alterations are uneven from one glomerulus to the other and as well as within the same glomerulus. The thickened walls are a consequence of the subendothelial deposition of light chains (110,156). There are a number of glomerular morphologic patterns, including mesangial, membranoproliferative, and crescentic (194–201), that precede the nodular glomerulopathy. Progression from one of these "early" patterns to a classic nodular glomerulosclerosis has been shown to occur over time using repeat kidney biopsies. A recognized "atypical" variant of LCDD shows peculiar light microscopic features and ultrastructural findings (202) simulating an immune complex–mediated disease. The light chain deposits, regardless of whether they are in renal or extrarenal sites, are always Congo red negative.

In patients with early LCDD, the glomeruli at times appear essentially unremarkable, and these cases may be incorrectly diagnosed as minimal change glomerulopathy if immunofluorescence staining for light chains is not performed (81,108,109,157,172). Recognition of this LCDD pattern is very challenging (109,110,172) and requires careful immunomorphologic evaluation and/or immunoelectron microscopy (108,157,172) to confirm the association of monoclonal light chain deposits with the capillary walls.

The extraglomerular changes may also be quite impressive. The tubular basement membranes may be thickened and tortuous, as a result of deposition of light chains, generally on the outer aspect of the tubular basement membranes (Figs. 22.8A and 22.9). In our experience, thickening of vessel walls by light chain deposits is seen in approximately 40% of LCDD cases. In half of LCDD patients with vascular changes, concentric thickening of the small and medium arteries accompanied by focal light chain deposits (172) creates a striking hyperplastic vasculopathy. The interstitial deposits are sometimes visible by light microscopy, where they form PAS-positive aggregates and are rarely associated with giant cell reaction. Light chain deposits can be found in many organs, including the lung, liver, small and large intestine, thyroid, prostate, pancreas, rectum, skin, spleen, and choroid plexus, among others (57,177,182,183,203).

Immunofluorescence

Deposition of monoclonal light chains can be seen along the peripheral capillary walls in the glomeruli, alongside the tubular basement membranes, and in the vessel walls (Fig. 22.10); the staining is usually diffusely linear and regular but, in some cases, can be interrupted or subtle (55,57,108,110,159,183). The pattern and intensity of staining depend on the amount and distribution of light chain deposits. In most cases of LCDD, the pathogenic light chain is of kappa isotype. No staining is noted for the other light chain, defining the monotypic nature of the labeling pattern. In most cases, there is also granular monotypic light chain mesangial staining in the glomeruli, but mesangial staining is rare without accompanying capillary wall staining. In a subset of these patients, there is also focal granular staining for the pertinent light chain in the interstitium proper. Staining for immunoglobulin heavy chains (IgG, IgM, and IgA) as well as complement components C3 and C1q are typically negative. Rare atypical cases of LCDD have been associated with positivity for complement components (204). In some cases, there is a discrepancy between the fluorescence staining and the demonstration of light chains by electron microscopy. In fact, there are a few reported cases of combined LCDD and light chain cast nephropathy, in which the monotypic light chain deposition has been demonstrated by immunofluorescence and no corresponding deposits were found ultrastructurally (89,156). Such

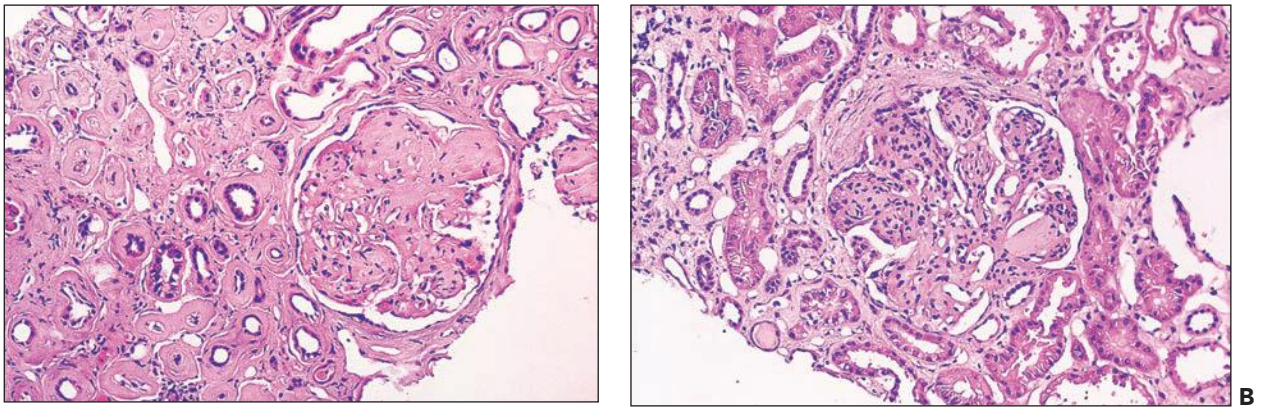

FIGURE 22.8 Light chain deposition disease. Nodular glomerulopathy characteristic of LCDD is evident in **(A)**. Note variation in cellularity, with hypercellular nodules present in **(B)**. (H&E, ×500.)

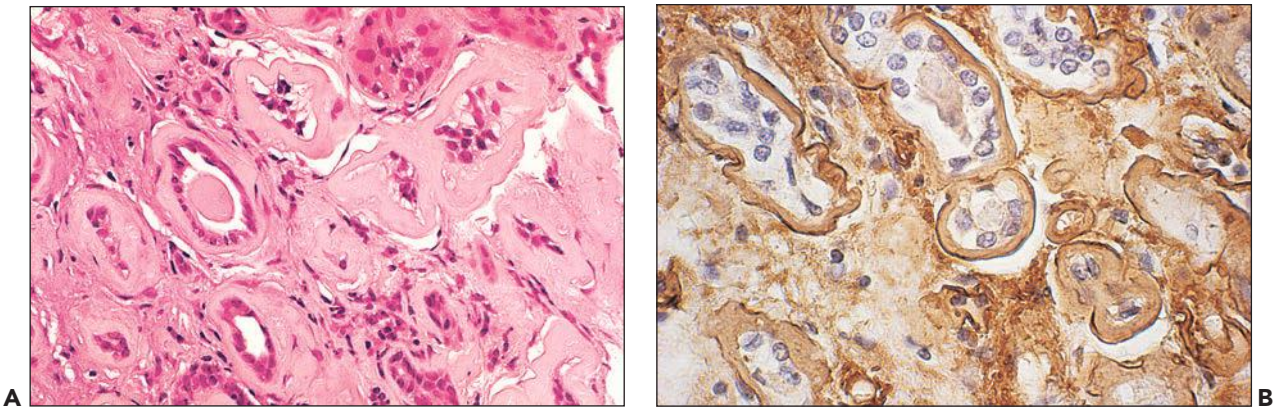

FIGURE 22.9 Light chain deposition disease. A: Thickened tubular basement membranes. (H&E, ×500.) **B:** κ Light chain deposition on the outer aspect of the tubular basement membranes. (Immunohistochemistry for κ light chain, peroxidase antiperoxidase stain, diaminobenzidine as marker, ×500.)

cases often accompany light chain cast nephropathy and are referred to as "LCDD by immunofluorescence only."

Generic antibodies to κ and λ light chains cannot detect all pathologic light chains deposited in the kidney in patients with LCDD. Only when a specific antibody is raised against the pathologic light chain can it be guaranteed that the light chain will be detected. Also, in patients with concomitant diabetes mellitus and LCDD, the abnormal light chains are frequently glycosylated to a degree that impairs their detection. The usual diffuse linear staining seen along peripheral capillary walls in diabetic patients may make it difficult to identify a monoclonal light chain. Immunogold electron microscopy is successful in labeling the abnormal light chains even when generic antibodies are used, attesting to the increased sensitivity of this technique (157). Immunofluorescence techniques have detected deposits in virtually every organ (183,203).

Electron Microscopy

Light chain deposition manifested by flocculent to granular to powdery electron-dense material can be seen in all renal compartments (Figs. 22.11 and 22.12), especially in cases with nodular glomerulosclerosis (advanced lesion). Visualization of light chain deposits may be challenging because the deposits may blend with the mesangial matrix and glomerular/tubular basement membranes. In the glomerular capillary walls, the deposits tend to form thin band-like powdery deposits along the lamina rara interna. Larger deposits may pool in the subendothelial regions. In rare cases, the electron-dense light chain material infiltrates the lamina densa, mimicking dense-deposit disease (108,109,205). At low magnification, the light chain deposits may simulate immune complexes (202). Powdery electron-dense deposits are also generally present along the outer aspect of the tubular basement membranes, along the Bowman capsule, and in the interstitium proper (Fig. 22.12A) (206,207). There may also be distinct deposition of light chains in the vasculature, where they tend to deposit in the intima and the basement membrane cuffs that surround individual medial myocytes (Fig. 22.12B). The light chain deposits may be subtle when the amount of light chain deposits is limited (108,109), and they may blend with the surrounding structures. In combined MIDD and cast nephropathy cases, the light microscopic alterations in the glomeruli and other

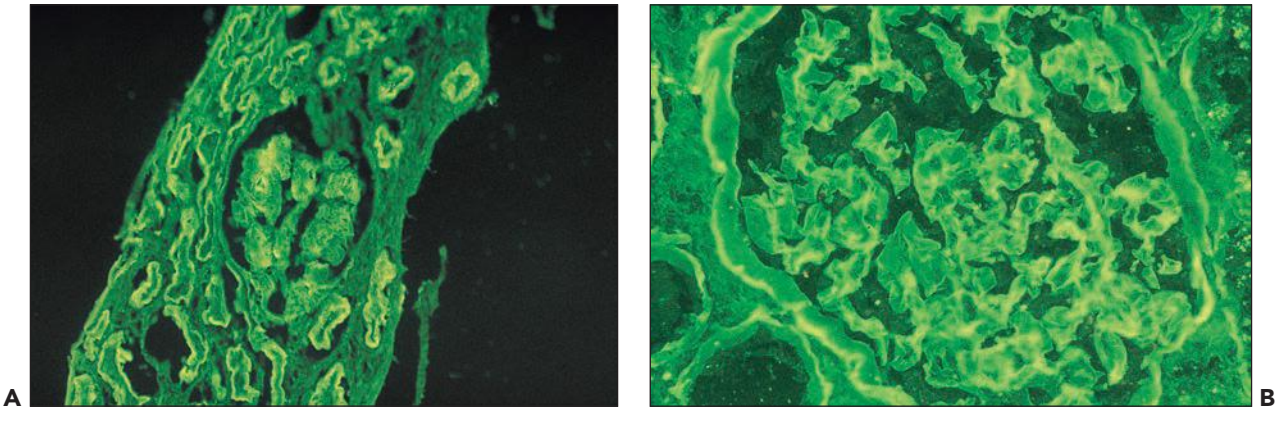

FIGURE 22.10 Light chain deposition disease. A: Low-power view showing linear staining along the tubular basement membranes and mesangial staining in the glomeruli for κ light chains. (Fluorescein, ×150.) **B:** Linear peripheral glomerular capillary wall staining and staining along the tubular basement membranes and Bowman capsule. Direct immunofluorescence for κ light chain. (Fluorescein, ×500.)

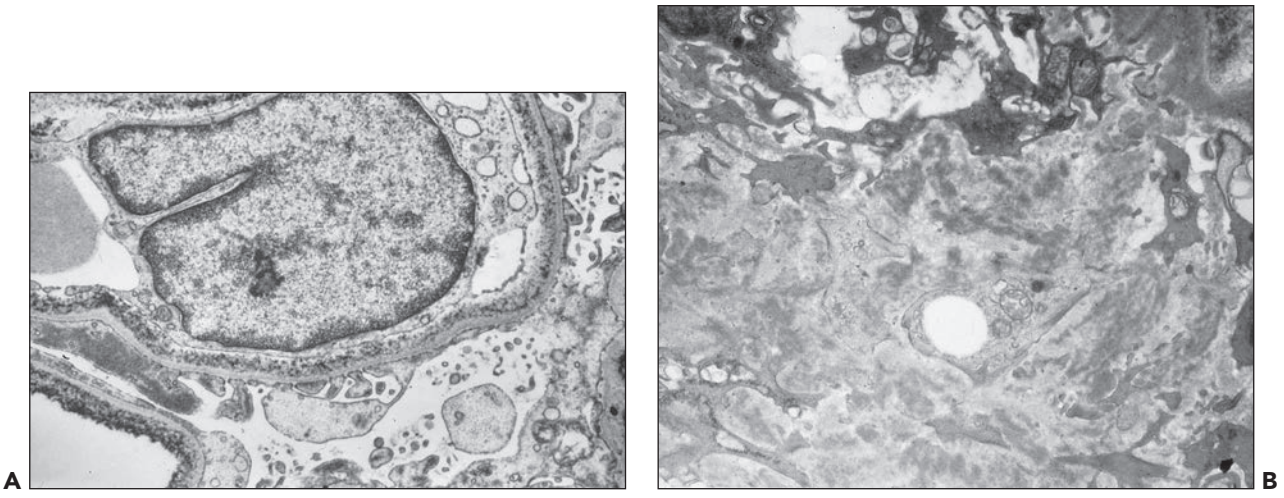

FIGURE 22.11 Light chain deposition disease. A: Continuous, punctate, subendothelial, electron-dense material (light chains) outlines the peripheral glomerular capillary wall. (Uranyl acetate and lead citrate, ×8500.) **B:** Mesangial light chain deposition. Transmission electron microscopy. (Uranyl acetate and lead citrate, ×9500.)

compartments may be subtle and easily missed, but combining the data obtained from immunofluorescence and electron microscopic evaluation should suffice to make the correct diagnosis (Fig. 22.13) (81,89,156).

Etiology and Pathogenesis

Conclusive evidence that the abnormal light chain protein is primarily responsible for light chain–associated disease has been provided from an in vivo model using mice injected with human BJ proteins from patients with LCDD. Solomon et al. (62) demonstrated nodular and diffuse light chain precipitates in the tubular basement membranes, mesangium, and vessel walls, recapitulating the key findings in the kidneys of these patients. Khamlichi et al. (49) confirmed these results.

Since then, the pathogenesis of LCDD has been scrutinized using renal biopsy material and an in vitro mesangial cell culture model. Studies on renal biopsies demonstrating platelet-derived growth factor–β (PDGF-β) and transforming growth factor–β (TGF-β) in the mesangium of patients with LCDD in various morphologic stages suggested a role for these two growth factors in the pathogenesis of this disorder (208). The in vitro model confirmed the findings in the renal specimens

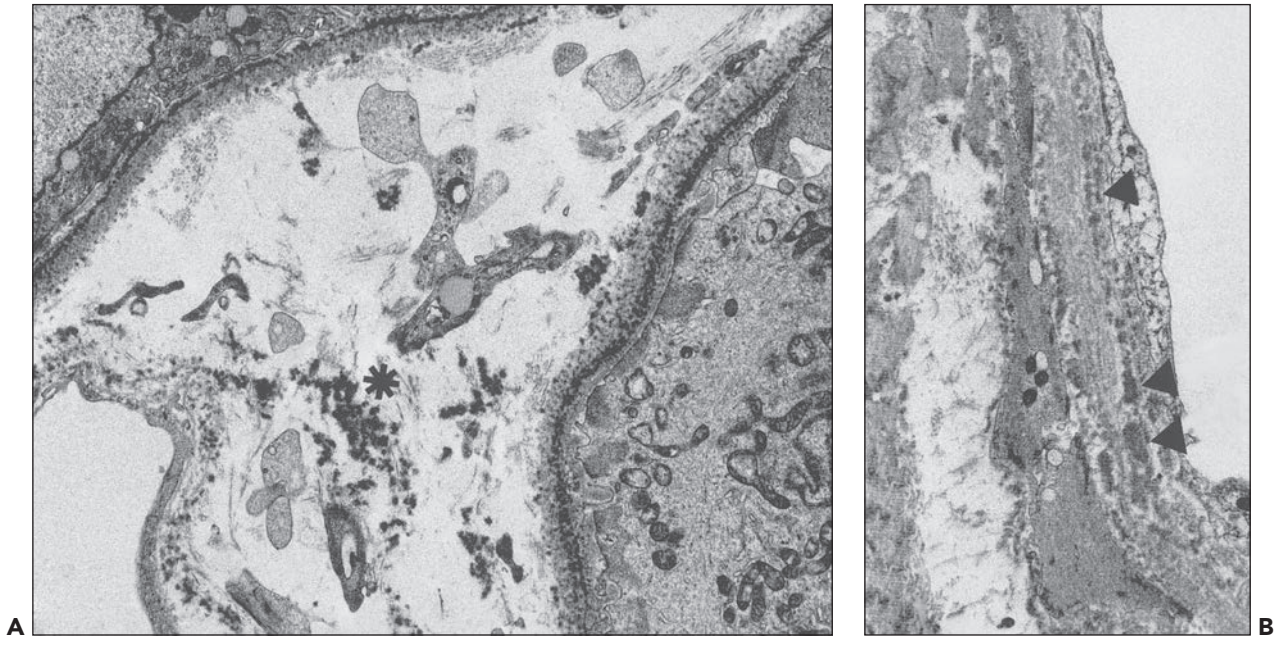

FIGURE 22.12 Light chain deposition disease. Punctate, electron-dense material along the tubular basement membranes and in the interstitium proper (*asterisk* in **A**) and along the vessel wall (*arrowheads* in **B**). Transmission electron microscopy. (Uranyl acetate and lead citrate.) (**A:** ×7500; **B:** ×9500.)

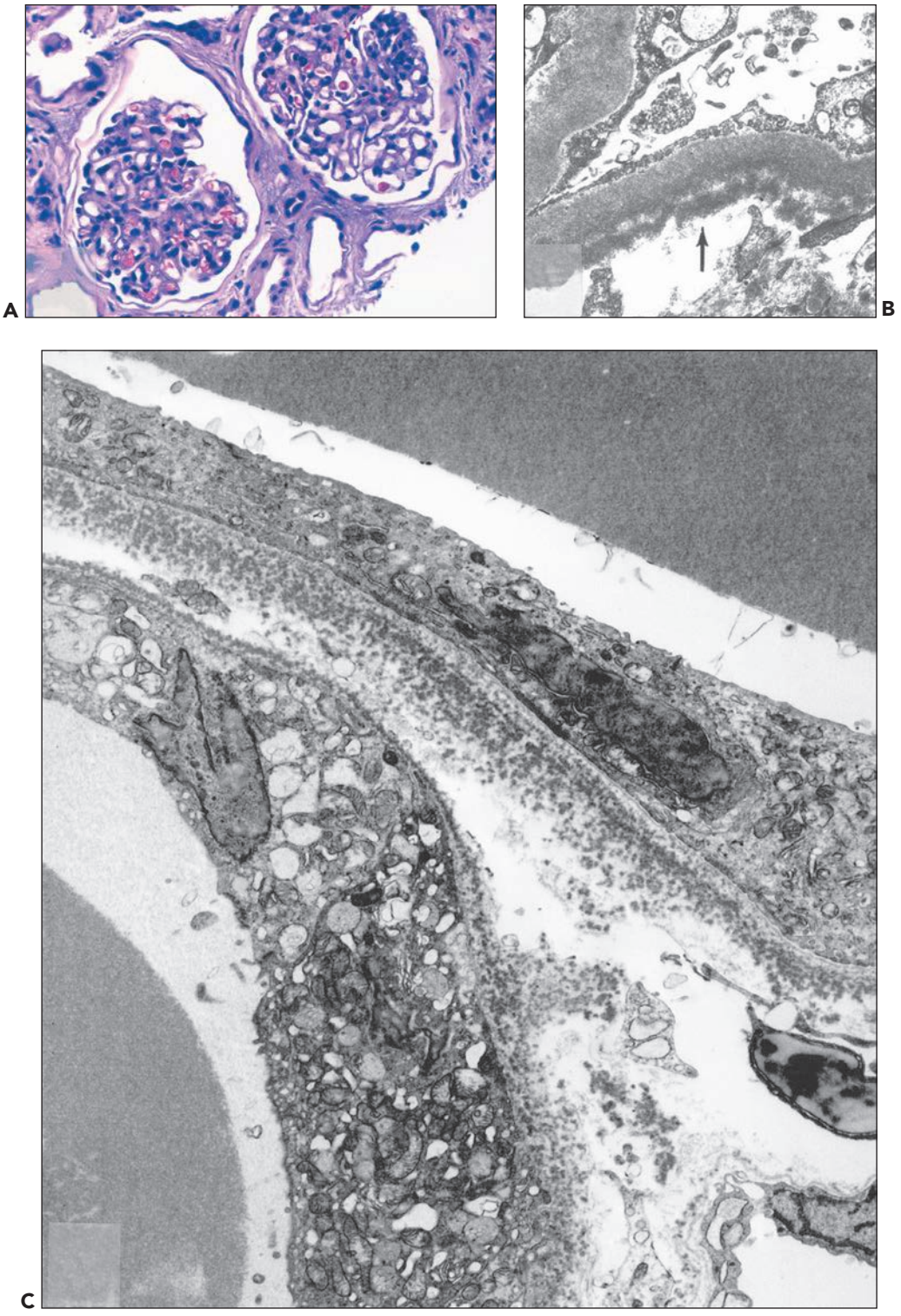

FIGURE 22.13 Light chain deposition disease and light chain cast nephropathy, combined. A: Glomeruli are unremarkable by light microscopy. (H&E, ×500.) **B:** However, ultrastructurally, deposition of light chain material is noted along the peripheral glomerular capillary walls (*arrow*) and (**C**) along the tubular basement membranes, associated with abundant distal tubular casts. **B, C:** Transmission electron microscopy. (Uranyl acetate and lead citrate.) (**B:** ×9500; **C:** ×7500.)

and showed that interactions between mesangial cells and light chains from these patients were crucial in the pathogenesis. Rat and human mesangial cells grown on cover slips and in a matrix with composition similar to that of an altered mesangial "milieu" and incubated with light chains purified from the urine of patients with LCDD recapitulated the biopsy findings (209–213). Human mesangial cells incubated with light chains purified from the urine of patients with LCDD transform from their usual smooth muscle to a myofibroblastic phenotype (214), endowing them with the necessary machinery to engage in active synthesis of extracellular matrix proteins. The initial events were activation of PDGF-β, leading to cellular proliferation through c-fos activation, and later TGF-β activation, resulting in matrix deposition (203,211–213). The in vitro model clearly demonstrated the crucial role of TGF-β in the production of extracellular matrix proteins by mesangial cells in LCDD (213). Tenascin is the main extracellular matrix protein in the nodules formed in the in vitro model as well as in the mesangial nodules in nodular glomerulosclerosis in LCDD (197,210,211) by transformed mesangial cells with a myofibroblastic phenotype (214). Tenascin is mainly degraded by matrix metalloproteinase-7 (MMP-7), with minor contributions by MMP-1 and MMP-3 (215). MMP-7 secretion by mesangial cells is impaired in LCDD, making tenascin degradation virtually impossible (216). The specific mechanism responsible for this has not yet been elucidated. The interactions between the light chains responsible for LCDD and mesangial cells occur through a receptor-mediated mechanism (217) (Fig. 22.14).

Although κ light chains are preferentially associated with LCDD, amino acid substitutions introducing hydrophobic residues in the exposed portions of the variable region of the light chains, usually in the CDR1 and CDR3 and less often CDR2 and FR regions, are associated with LCDD, regardless of light chain subtype (218,219). Some light chains with posttranslational glycosylation have also been associated with LCDD (220).

Treatment, Course of the Disease Process, and Prognosis

The outcome in LCDD remains uncertain and depends on how early in its course the disease is detected. Overall, patient survival at 1 and 5 years (89% vs. 70%), respectively, is better than renal survival (67% at 1 year vs. 17% at 5 years) (156). Approximately one third of the LCDD patients without diagnostic features of myeloma develop overt myeloma during the course of the disease, affecting survival adversely. Extrarenal deposits can be asymptomatic or associated with organ damage (182,203).

Treatment is aimed at controlling and reducing the abnormal light chain production by the clone of plasma cells. Chemotherapy with melphalan and prednisone has been employed with success in patients with LCDD and myeloma (221), but the benefit of chemotherapy has been debated when the bone marrow findings are inconclusive, especially if a monoclonal protein cannot be demonstrated in serum or urine (220). Chemotherapy is most effective in cases with a serum creatinine lower than 2 mg/dL. The severity of the underlying plasma cell dyscrasia and the degree of renal failure at presentation, as expected, significantly affect prognosis (89). Bone marrow transplantation has been used, especially in cases detected early, before compromise of other organs has occurred (220–223). Those patients with overt myeloma do much worse than those with no detectable paraprotein/proliferation of plasma cells and systemic manifestations. However, even in those cases with poor response, progression to end-stage renal disease may take several years.

Early diagnosis and aggressive therapy appear to offer the best hope for these patients in terms of preservation of renal

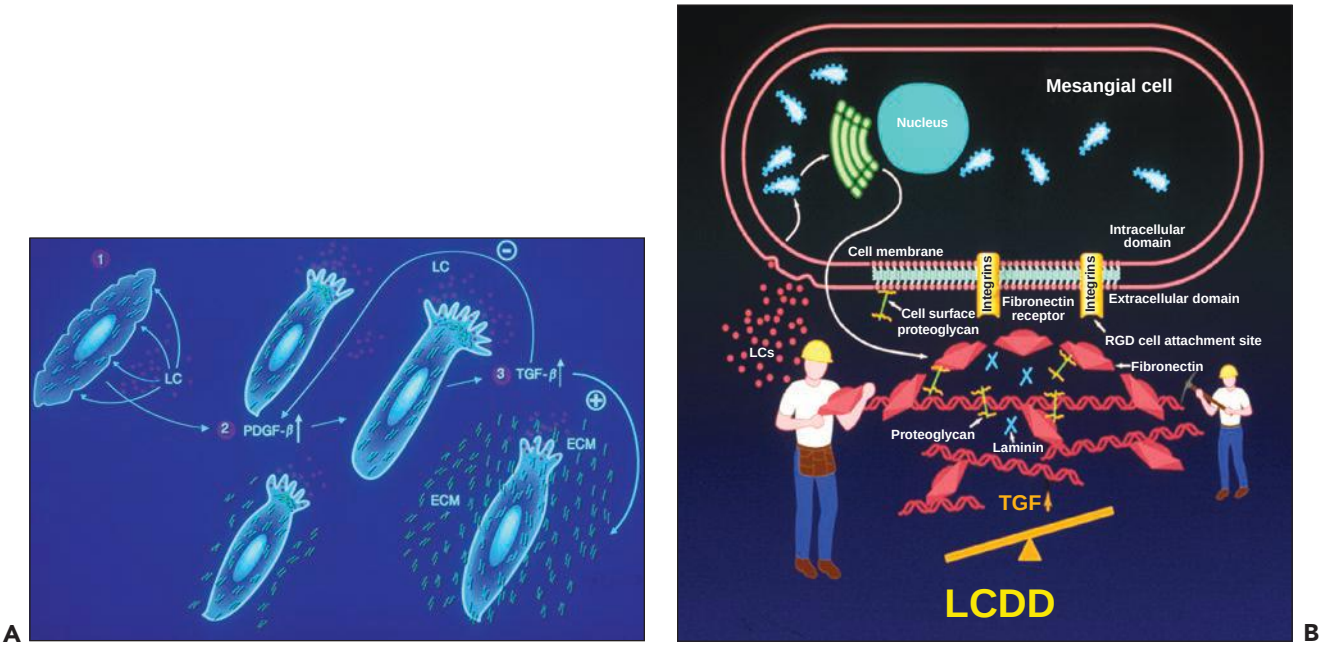

FIGURE 22.14 **Light chain deposition disease. A:** Glomerulopathic light chain-mesangial cell interactions with interplay of growth factors—PDGF-β and TGF-β. **B:** End result in LCDD. Increased mesangial matrix as a result of TGF-β production and decreased activity of metalloproteinases.

function and overall survival (223). High-intensity chemotherapy followed by stem cell rescue and bone marrow transplantation has been used in several trials, resulting in increased patients' survival and improvement in renal function (224), and has become a standard therapy (223).

There are a few reports of resolution or disappearance of nodular glomerulosclerosis after therapy in patients with LCDD, suggesting that early intervention can reverse renal damage in these patients (225,226). New therapies aimed at targeting key steps in the cascade of events involved in glomerular and interstitial damage are to be designed to be used in combination with chemotherapy and other therapeutic interventions that control light chain production (195).

Transplantation in Light Chain Deposition Disease

Renal transplantation has been successful in patients with disease confined to the kidneys (227,228). If the production of the precursor protein is not controlled or eliminated before transplantation, the disease will recur in the transplanted kidney. Because complete control of the plasma cell dyscrasia is virtually impossible, recurrence is inevitable, and recurrence occurs in more than 50% of the patients 8 to 48 months after transplantation and may eventually lead to graft loss (227–231). In a series of patients documenting the outcome of seven LCDD patients that were transplanted, recurrence occurred in five of the allografts (227). Recurrence is not always associated with rapid loss of the transplanted kidney; the median time to reach end-stage renal failure was 33.3 months. One of these transplanted patients was alive with no evidence of recurrence 13 years after transplantation (228). Therefore, renal transplantation can be used to improve quality of life in certain patients but not as a long-term solution in the majority. Unfortunately, even when it is believed that control of the plasma cell clone has been achieved, it can reemerge, followed by light chain deposition. LCDD can recur without clinical evidence of underlying myeloma (231). There is a case report of de novo LCDD arising in a transplanted cadaver kidney 16 years after transplantation (232).

Heavy Chain Deposition Disease and Light and Heavy Chain Deposition Disease

Clinical Presentation and Laboratory Findings

Heavy chain disease (HCD) is a disorder characterized by the production of monoclonal immunoglobulins with truncated heavy chains and no associated light chains. Abnormal heavy chains circulate in the blood of patients with this disorder, and these paraproteins must be identified in the serum and characterized to make a definitive diagnosis. HCD involving three immunoglobulin classes has been described: α-HCD is the most common of the three, followed by γ-HCD and then μ-HCD. Patients with α-HCD present with malabsorption and protein-losing enteropathy; renal disease is not a significant clinical manifestation of this condition and only occurs sporadically. Approximately 150 cases of γ-HCD have been reported, and it is a heterogeneous disorder, which some believe does not justify its diagnosis as a single entity (233). This disease usually manifests with lymphadenopathy, splenomegaly, and constitutional symptoms. Palatal edema and uvula swelling, initially thought to be characteristic of the disease, are only noted in approximately 10% to 15% of all patients with γ-HCD (214). Skeletal involvement occurs in approximately 30% of these patients, and differentiation from myeloma and Waldenström macroglobulinemia (WM) may be difficult on clinical grounds (233). γ-HCD is the most common type of HCD associated with renal manifestations (233). There is an overrepresentation of γ3 and γ4.

A minority of patients with HCD develop tissue deposits, an entity known as HCDD. Bence Jones proteinuria is common. HCD and cast nephropathy have been documented to coexist (151). There is not much experience with μ-HCD and renal disease (171,233,234). HCDD is mainly associated with γ (γ1- and γ3-subtypes) (235,236) and rarely with α-heavy chains (237).

A few hundred patients with HCDD have been described in the literature (155,233). There is no sex predilection for HCDD. Patients with HCDD and renal disease tend to be younger than those with LCDD by a few years (mean age 53 to 55 years), but some patients develop HCDD as early as the third decade of life (156,171). The clinical presentation is similar to that of LCDD patients. Hypertension, nephrotic syndrome, microhematuria, and renal insufficiency are usually present at the time of the renal biopsy (171). Hypocomplementemia is not uncommon in HCDD and correlates with the presence of 1 or 3 subtypes of γ heavy chain (171,236). Signs of complement activation with C3 and C1q deposition in the kidney represent an additional feature of this condition (156,171), mostly when γ1- and γ3-chains are involved (190). Serum hypocomplementemia may also be noted. Hypertension and hematuria appear to be more common in HCDD than in LCDD (171). In some patients with HCDD, a monoclonal component is not detectable in serum and/or urine. Whereas most patients with HCDD have a monoclonal gammopathy, only a few meet the minimal criteria for myeloma (89), at least at presentation.

It is likely that HCDD is underdiagnosed in renal biopsies, and therefore the incidence of this condition is probably underestimated. For example, two recent autopsy series of patients with myeloma (82,88,187) failed to identify any patients with HCDD, suggesting that the overall knowledge about this disease and methods available to diagnose it remain less than optimal. Patients with findings in their renal biopsies that are suggestive of deposition disease with negative stains for κ and λ by immunofluorescence should be worked up for HCDD.

Some cases are combined LCDD and HCDD, termed LHCDD (145), and γ is the most frequent heavy chain component in these patients. Both κ and λ light chains have been found in the LCDD component of these cases (156). The combined variety, known as LHCDD, occurs in older patients—by approximately 10 years—than patients with LCDD or HCDD alone (156). Overall renal function is more significantly impaired at presentation when the two deposition disorders coexist, with an average serum creatinine in the neighborhood of 5 mg/dL. However, proteinuria is usually in the range of 3 g/24 h. The diagnosis of LHCDD requires careful pathologic evaluation of the renal biopsies and a high index of suspicion (156). The diagnosis cannot be reached by light microscopy alone; immunofluorescence with the use of specific antisera is required. Ultrastructural findings may provide confirmatory evidence of light/heavy chain deposition in various renal compartments.

Gross Pathology

There are no studies describing specific gross findings in patients with HCDD.

Light microscopy

In the great majority of the reported cases of HCDD, nodular glomerulosclerosis with features identical to those seen in association with LCDD has been identified (Fig. 22.15) (155,234–238). Crescents were described in four of nine cases, involving 11% to 75% of the glomeruli in one series (155,237). The broad spectrum of glomerular lesions described in LCDD has not been documented in HCDD. A single case of intracapillary proliferative glomerulonephritis has also been reported (239). Crescents are more common in HCDD. Tubular basement membrane deposits with no glomerular or vascular heavy chain deposition have been reported (155). Congo red stain is always negative.

Immunofluorescence

Staining for only one heavy chain class is positive, whereas staining for both light chains is negative. Cases with γ1, γ3, γ4, α, and μ chain deposits have been reported, and γ chain deposits are the most common (156). Thus, cases of HCDD are diagnosed by finding linear staining of renal basement membranes with antisera to one immunoglobulin class (IgG in cases of γ heavy chain, IgA in cases of α heavy chain, or IgM in cases of μ heavy chain), but negativity for both kappa and lambda light chains. The distribution of staining is similar as that for LCDD (Fig. 22.16), but the degree of staining along tubular basement membranes is generally less than in LCDD. The staining for the heavy chain components varies from a predominantly linear to a much less common granular pattern (89,156,178). The heavy chain deposits usually display a uniform, continuous pattern of deposition at the various sites responsible for the linear fluorescence staining pattern noted in the great majority of the cases. Specific antibodies for constant regions of the heavy chain molecule that are deleted in this condition (uniformly lacking C_H1 and in some cases also C_H2) can be used to demonstrate the absence of these components and confirm the diagnosis (89,125,182,194,195,238). In cases of γ HCDD, staining for the gamma chain subtypes (1–4) will help to confirm the diagnosis by identifying a single gamma subtype. As previously noted, granular C1q and C3 may be observed in the same distribution as the heavy chain. Extrarenal deposits of heavy chain components have been reported in the pancreas, thyroid, striated muscle, and liver but are less frequent than in LCDD (89,156).

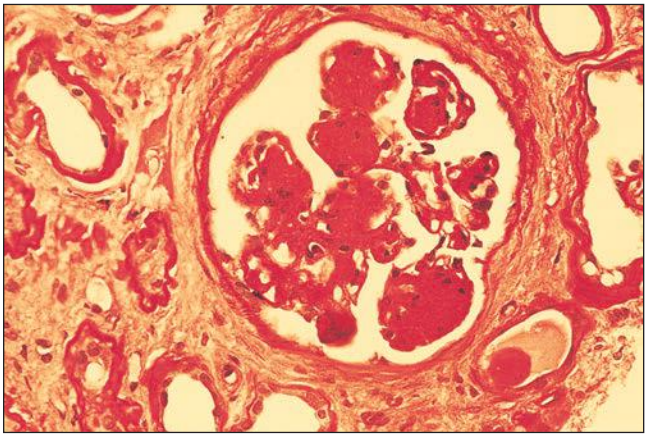

FIGURE 22.15 Heavy chain deposition disease. Morphologic findings in HCDD are identical to those in LCDD, with nodular glomerulopathy as the typical pattern. (H&E, ×500.)

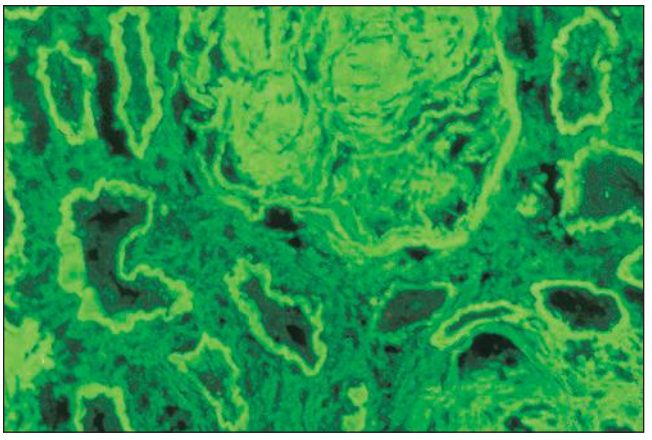

FIGURE 22.16 Heavy chain deposition disease. Intense peripheral capillary wall and mesangial staining, associated with linear tubular basement membrane staining. Direct immunofluorescence staining for μ heavy chain. (Fluorescein, ×350.)

Electron Microscopy

The ultrastructural findings in HCDD are similar to those in LCDD in most instances. The heavy chain deposits can be subtle or massive. Overall, heavy chain deposits are variable in quantity and distribution in the various renal compartments. In one case, the deposits were described as fibrillary, consisting of 13- to 18-nm-diameter fibrils without periodicity, judged ultrastructurally to be different from those found in fibrillary glomerulopathy. The fibrils exhibited various lengths, with the shorter ones having smooth walls and the longer fibrils exhibiting a "barbed-wire" appearance (240). This case appears to represent an unusual morphologic manifestation of μ-chain HCDD. The patient had no clinical manifestations of Waldenström macroglobulinemia. Another μ-HCDD had massive fibrillary deposits in the mesangium that varied from 16 to 18 nm in diameter and were determined not to be compatible with the material seen in fibrillary glomerulopathy by electron microscopic criteria (234). These two cases suggest that there may be a variant of HCDD with peculiar fibrillary ultrastructural appearance, but this deserves further consideration.

Etiology and Pathogenesis

The deletions in the domains of heavy chain portion of the immunoglobulin molecule (i.e., C_H1, C_H2, and very rarely, hinge region) result in premature secretion of the heavy chain into the circulation, and these structurally abnormal heavy chains are deposited in target organs, including the kidneys (241). The nascent γ heavy chain protein normally is retained in the endoplasmic reticulum during IgG assembly by binding of its C_H1 domain (and to some extent C_H2 and hinge region) to chaperone protein, heavy chain–binding

protein. The specific mechanisms involved in the pathogenesis of the renal alterations that occur in this disorder have not been elucidated.

Treatment, Course of the Disease Process, and Prognosis

The therapy employed is similar to that of LCDD, and the results appear to be comparable (198). However, there are no controlled trials addressing the therapy and management of patients with HCDD. Nevertheless, it appears that, based on anecdotal experience, the overall outcome is poor in terms of renal and patient survival. One patient had γ-HCDD for 10 years and subsequently developed γ AL amyloidosis (242).

Transplantation in Heavy Chain Deposition Disease

The experience with transplantation in this disease is limited. One transplanted HCDD patient developed recurrent disease approximately 1.5 years after transplantation, so the fate of renal transplants in this condition may be similar to the more extensive experience in LCDD (228,229).

Differential Diagnosis for Heavy Chain Deposition Disease and Light and Heavy Chain Deposition Disease

Because the light microscopic appearance of LHCDD is so variable, the differential diagnosis includes many diseases, and it must be differentiated from minimal change disease in those cases where the glomeruli appear essentially normal, from mesangial proliferative glomerulonephritis when mesangial proliferation is present, and from membranoproliferative glomerulonephritis, including dense-deposit disease; also, crescentic glomerulonephritis must be distinguished from the proliferative variants of LCDD (108). When considering the most characteristic expression of LCDD and HCDD—nodular glomerulosclerosis—the main differential diagnosis is diabetic nephropathy or "idiopathic" nodular glomerulosclerosis (244). In most instances, demonstration of monoclonal light or heavy chain determinants in the proper histopathologic setting is the essential diagnostic finding. Careful attention to the tubulointerstitial and vascular compartments for light chain deposits is also imperative, as there is a subset of LCDD patients with no glomerular light chain deposition and only tubulointerstitial manifestations of this disease (108,156). A diagnosis of γ-HCDD can be confirmed by using antisera to the IgG subtypes [1–4] and demonstrating staining for a single subtype. It is also helpful to identify deletions of C_H1 and, in some cases, C_H2 using specific antisera to the constant domains (C_H1 to 3) of the γ heavy chain. Rarely, amyloidosis needs to be ruled out, especially in cases with nodular glomerulopathy. In the great majority of these situations, immunofluorescence and electron microscopy suffice, and a solid, unequivocal diagnosis can be obtained. Selective use of immunoelectron microscopy is indicated when the usual diagnostic techniques do not provide enough data to establish an unequivocal diagnosis (157). In the experience of one of the authors (G.A.H.), approximately 5% of the cases require an extended workup beyond routine light, immunofluorescence, and ultrastructural evaluation (157).

Other Entities in the Differential Diagnosis

Glomerulonephritis Associated With Monoclonal IgG Deposits and Monoclonal Gammopathy of Unknown Significance

This entity was first recognized as a specific entity and given its name in 2004 (245). An additional case was reported the same year (246). There are approximately 60 cases of this entity reported in the literature. By light microscopy, these cases exhibit proliferative glomerular changes, most commonly resembling membranoproliferative glomerulonephritis, type I (in about 60% of these cases) (245–250) (Fig. 22.17A). The glomerular electron-dense deposits are granular and nonorganized, ultrastructurally resembling immune complex–mediated glomerulonephritis (Fig. 22.17D), but exhibit a unique immunofluorescence profile; they contain monoclonal IgG (predominantly IgG3) and monoclonal light chains (Fig. 22.17BC), either kappa or lambda. The electron-dense deposits are characteristically located subendothelially and also in mesangial areas. In cases with predominantly subepithelial immune complexes, the light microscopic pattern was that of a membranous nephropathy (247,248). Clinically, these patients present with varying degrees of proteinuria (100% of patients reported), which is most often nephrotic range; microhematuria (about 60% of the patients); and renal insufficiency. Another subgroup of these patients exhibit morphologic features that are similar to dense-deposit disease (251).

A monoclonal serum or urine protein is identified in approximately half of the patients; however, only rare patients are associated with myeloma or B-cell lymphoproliferative disorders. The largest series of 37 patients was published in 2009 with only 1 of the patients revealing evidence of myeloma and 1 with AL amyloidosis (250). There are 21 additional patients reported in the literature. Only 3 of these of the 16 patients with available data had a monoclonal protein in the serum and/or urine (245,249,252).

Treatment has consisted of a combination of regimens depending on the particulars of a given case. Renin-angiotensin (RAS) blockade alone was used in about 30% of the patients. Immunomodulatory therapy with or without RAS blockade has been the most commonly used treatment (249). Rituximab has been reported in another series of these patients with satisfactory results (253). It is fair to state at the present time that the best therapy has not been defined due to the small number of patients that have been documented with this condition and the lack of well-defined therapeutic trials.

Short follow-up (<3 years) reported in the largest series demonstrated approximately one third of the patients with complete or partial recovery, another 40% or so with persistent renal dysfunction, and the remainder progressed to end-stage renal disease. One of the patients in this cohort without a monoclonal spike at the time of presentation developed a hematologic malignancy. This entity has been reported to recur in the transplant, where some cases have responded favorably to rituximab (254).

It is important to differentiate these patients from others with monoclonal light chain–related renal disease because this entity does not appear to be a precursor of or significantly associated with an underlying plasma cell dyscrasia, except in rare instances.

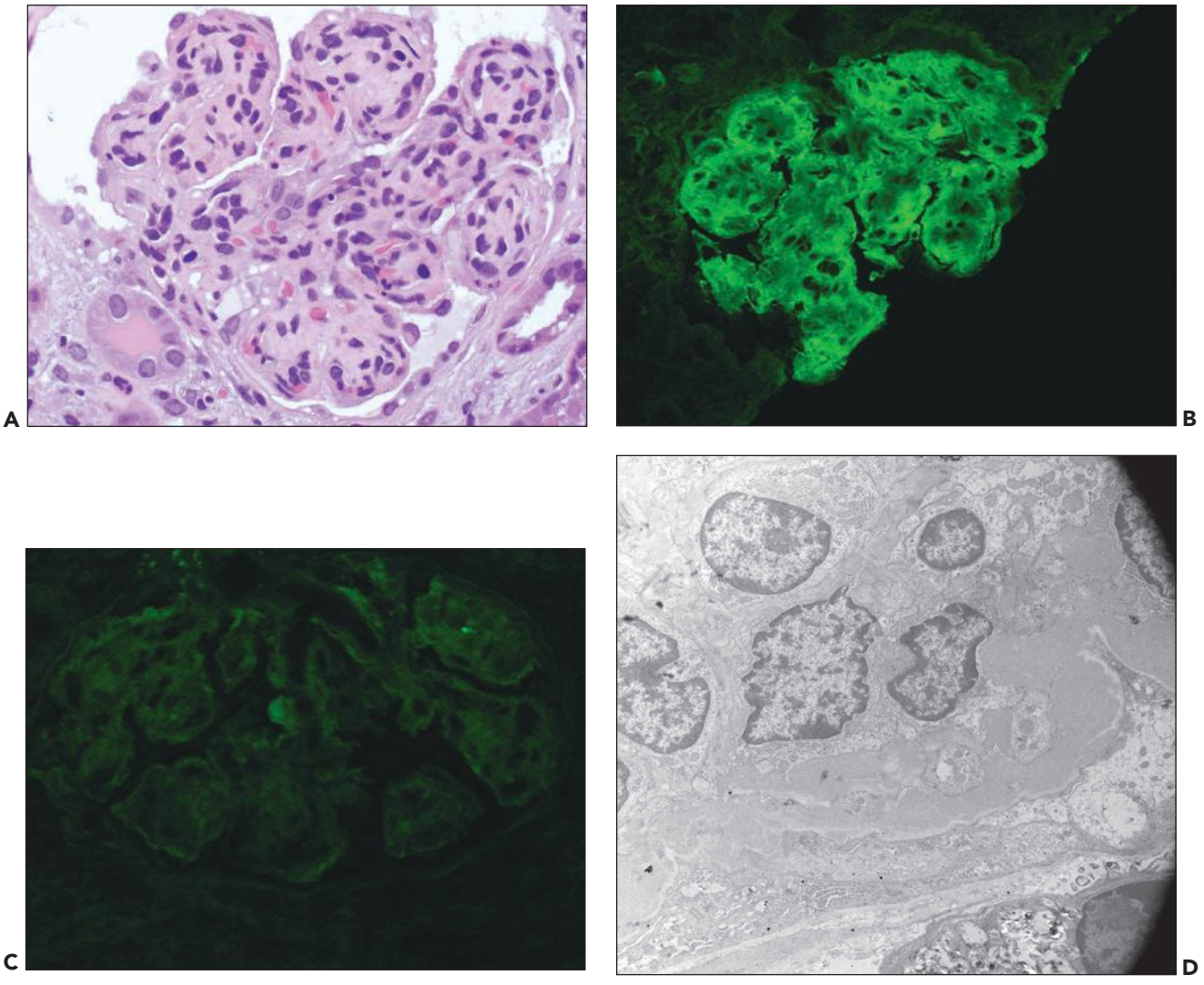

FIGURE 22.17 Proliferative GN with monoclonal deposits. A: Light microscopy with membranoproliferative appearance. (H&E, ×500.) **B:** Staining along the peripheral capillary walls and in the mesangium. (Immunofluorescence, kappa, ×500.) **C:** No glomerular staining. (Immunofluorescence, lambda, ×500.) **D:** Large subendothelial granular electron-dense deposits. (Transmission electron microscopy, uranyl acetate, and lead citrate, ×12,500.)

Glomerulonephritis With C3 Deposits Associated With Monoclonal Gammopathy of Unknown Significance

The first reported association between isolated C3 deposits and monoclonal gammopathy was by Appel et al. (249). Similar cases have been reported by several groups (251,255–258). In a group of 81 hepatitis-negative patients with membranoproliferative glomerulonephritis associated with monoclonal light chain deposits, 28 had evidence of monoclonal gammopathy, and 3 had isolated C3 deposits. Nasr et al. (256) reported that 4 adults in a series of 32 patients with C3 deposits and dense-deposit disease had a history of plasma cell dyscrasia. Finally, a series of six patients with MGUS and a glomerulonephritis with isolated C3 staining has been reported more recently (255). These patients range from 40 to 70 years of age, and they presented with hypertension and significant proteinuria with full-blown nephrotic syndrome in three patients. Hematuria was present in the six cases, and all had evidence of renal insufficiency. The renal biopsies demonstrated variable glomerular proliferative features, exudative changes, and some thrombi in capillary spaces. By immunofluorescence, only C3 deposits were apparent along peripheral capillary walls and in the mesangium, indistinguishable from C3 glomerulopathy. No monoclonal light or heavy chain staining was present. Ultrastructurally, there were subepithelial, intramembranous, and mesangial electron-dense deposits. The ultrastructural findings were interpreted as showing overlapping features between type II and III MPGN. In one patient, a repeat renal biopsy demonstrated glomerular monoclonal lambda light chain deposits that colocalized with the C3 in the mesangium and along the peripheral capillary walls. Some of these cases have exhibited features morphologically consistent with dense-deposit disease (258).

Patients were treated with high-dose dexamethasone, either alone or with melphalan or cyclophosphamide. One

patient progressed to myeloma after 11 years of follow-up. Overall, the renal outcome of these patients is poor with progression to end-stage renal disease in most cases (about 80%). Early therapy to control the plasma cell clone may be indicated in at least some of these patients (251).

Isolated C3 glomerular deposition, resembling C3 glomerulopathy, likely represents an unusual complication of plasma cell dyscrasias related to the activation of the alternative complement pathway by the monoclonal immunoglobulins. Some of these monoclonal light chains have been shown to interfere with inhibitors of the alternative pathway, such as factor H. The association of C3 glomerulopathy with MGUS, rather than overt, high plasma cell mass myeloma, suggests that prolonged alternative complement pathway activation by the monoclonal immunoglobulins is required for the development of other more advanced glomerular lesions where the monoclonal light chains may appear in renal tissue (259–261).

Thus, monoclonal gammopathy should be considered in the differential diagnosis of patients with MPGN. Some of these cases represent examples of LCDD and/or HCDD in stages prior to nodular glomerulosclerosis (109,161,257,258). In these instances, the typical linear immunofluorescence staining along the peripheral capillary walls and in the mesangium, and in outlining tubules and sometimes in vessels, together with punctate, electron-dense material indicative of light chains in the various renal compartments may provide solid diagnostic information. Other cases, however, may represent either proliferative glomerulonephritis with monoclonal IgG deposits or C3 glomerulopathy. Their unique immunofluorescence profiles and ultrastructural features allow differentiation from LCDD and HCDD.

Additional entities to be considered in the differential diagnosis of the above conditions are immunotactoid glomerulopathy and type I (monoclonal) cryoglobulinemic nephropathy. Immunotactoid glomerulopathy has characteristic microtubular structures with variable diameter, but generally in the 30- to 50-nm range identified ultrastructurally. In cryoglobulinemic nephropathy, intracapillary thrombi may be present by light microscopy, and the typically paired and short microtubular structures that characterize cryoglobulins provide a clue at the ultrastructural level. However, there is heterogeneity in the electron microscopic appearance of cryoglobulins, and intraluminal thrombi are not always present, making confirmation of this diagnosis difficult in some cases. Clinical correlation (usually markedly decreased C4 among others) and search for cryoglobulins in the serum may provide valuable confirmatory data. Patients with either proliferative glomerulonephritis associated with monoclonal IgG deposits and those with MGUS and isolated C3 deposits exhibit a significantly lower incidence of dysproteinemia than those with either monoclonal cryoglobulinemic nephropathy or immunotactoid glomerulopathy, making this distinction clinically useful for patient management and prognosis (255).

Plasma Cell Dyscrasias Associated With Crystalline Inclusions in the Glomeruli

Historical Perspective

In 1949, Neuman (262) described groups of small rhomboid crystals distending and deforming the Bowman capsule in the great majority of the glomeruli, along with similar structures in the proximal tubular cells in the kidneys from a patient with myeloma in an autopsy. Similar aggregates of crystals were identified in the lungs and in neoplastic plasma cells in the bone marrow. This initial report was followed by a second autopsy report by Sickel (263) 10 years later of a patient with enlarged kidneys, which on microscopic examination revealed crystalline inclusions in the glomeruli, along with some in the tubular cells. A diagnosis of clinically unsuspected multiple myeloma was made at autopsy. Carstens in 1989 reported a third case with numerous similar inclusions in parietal, visceral, endothelial, and mesangial cells in the great majority of the glomeruli (264). Since then, only rare case reports of this entity have been documented in the literature. While some of these may be included in cases with Fanconi light chain–associated tubulopathy, it is a fact that some patients only present with glomerular clinicopathologic manifestations.

Clinical Presentation and Laboratory Findings

While the finding of crystalline inclusions in the cytoplasm of tubular cells is a characteristic finding in light chain–associated Fanconi syndrome (discussed previously), and this entity may be sometimes associated with similar inclusions in the glomeruli, macrophages, and plasma cells, a similar entity exists where the crystalline structures are identified predominantly and, in some cases exclusively, in glomerular cells and in the matrix of the Bowman capsule. It is important to recognize this entity, as this may be the presentation of a patient with unsuspected myeloma. In these cases, the clinical presentation is typically proteinuria of variable degree and renal insufficiency, rather than Fanconi syndrome. In at least one reported case, the patient developed renal failure attributed to the glomerular process (264).

This entity is even more rare than Fanconi-associated proximal tubulopathy, with fewer than 10 cases reported in the literature. One of the authors (G.A.H.) has seen three of these cases in biopsy specimens in consultation. One of these cases (265) presented with a clinical picture of thrombotic microangiopathy. The case reported by Carstens was associated with extrarenal amyloidosis diagnosed in a carpal tunnel specimen (264). Another unique feature of this last case is that the glomerular inclusions were detected months before the diagnosis of myeloma was made. In the consultation cases, one had a diagnosis of myeloma, while in the other two, the renal biopsy findings indicated the presence of an underlying plasma cell dyscrasia that was later confirmed.

Gross Pathology

The two reported cases with gross pathologic data derived from the evaluation at autopsy revealed enlarged kidneys (263,264). In one of the cases, it was reported that each kidney weighed 396 g, more than double the size of normal kidneys.

Light Microscopy

The main findings are in the glomerular compartment where there is generally mesangial expansion and there may be prominent parietal and epithelial, as well as endothelial cells. The cytoplasm of affected cells is characteristically swollen and sometimes appears vacuolated with rectangularly/rhomboidal-shaped empty spaces with angulated borders. The size of the crystalline inclusions is variable, some being quite large. The cellular nuclei may be displaced to the periphery. The one case with clinical manifestations of thrombotic microangiopathy exhibited capillary thrombi composed of crystalline structures,

variably (frequently completely) occluding the lumina but no fibrin (265). The findings are somewhat similar to what is seen in the entity known as "crystal-storing histiocytosis" (152,153); however, the glomerular localization of the inclusions makes this particular entity unique.

Immunofluorescence

These cases exhibit light/heavy chain restriction. Light chain–associated cases have been related to kappa light chain related and the heavy chain restricted are gamma heavy chain associated. However, routine immunofluorescence evaluation may be negative. Pronase digestion may be of value in these cases, as it is in cases of Fanconi syndrome–related tubulopathy (159).

Electron Microscopy

The ultrastructural appearance of the glomerular inclusions is similar to those seen in the cytoplasm of tubular cells in cases of proximal tubular damage with intracytoplasmic inclusions associated with Fanconi syndrome (266).

Etiology and Pathogenesis

The light and heavy chain–related deposits result from in situ intracytoplasmic incomplete catabolism of these proteinaceous materials in various glomerular cell types. Peculiar physicochemical structures of the involved circulating immunoglobulin components are most likely the main reason for the genesis of the inclusions. Likely, the inability of lysosomes to completely catabolize the immunoglobulin precursors resulted in the formation of the crystalline inclusions.

Differential Diagnosis

The findings are pathognomonic and should not be confused with any other entity. It is important that this entity not be confused with a metabolic disorder. Demonstrating light or heavy chain restriction associated with the unique intracytoplasmic structures solidifies the diagnosis. This may not be easy, as the immunofluorescence workup may be negative.

Treatment, Course of Disease, and Prognosis

Due to the small number of cases and lack of proper follow-up, there are no controlled studies regarding treatment, course of disease, or prognosis.

AMYLOIDOSES

AL and AH amyloidoses (formerly termed "primary amyloidosis" and "amyloidosis associated with multiple myeloma") are included in this chapter firstly because these diseases are associated with a paraprotein and secondly because the amyloid fibrils that cause them are derived from immunoglobulin light (AL) or heavy chain (AH). Other types of amyloidosis, such as AA amyloidosis (formerly "secondary"), familial/hereditary amyloidoses, dialysis-related amyloidosis, and localized types that are not related to myeloma or paraproteinemia, are also discussed in this chapter because they have pathologic and pathogenetic similarities to AL and for historical reasons.

Definition

Amyloidosis comprises a group of protein folding disorders of diverse etiology, in which deposits of abnormally folded proteins share unique staining properties and a fibrillar ultrastructural appearance (267). The process of amyloid formation is toxic to tissues, and the resulting deposits ultimately lead to tissue destruction and progressive disease. Despite the diversity of amyloid proteins, all types of amyloid share a β-pleated sheet secondary structure that confers their diagnostic staining characteristics as well as their great stability under physiologic conditions (267). By electron microscopy, amyloid deposits consist of rigid, nonbranching fibrils that are on average 7.5 to 10 nm in diameter (267). However, the term "amyloid" was first coined by Schleiden in 1838 to describe a normal constituent of plants! He used an iodine-sulfuric acid staining reaction, first described in 1814, to identify starch and related compounds through the development of a blue or violet color (reviewed in (268–272)). In 1839, botanists began using this reaction to identify cellulose, a compound related to starch that, like starch, is made up of glucose repeating units (reviewed in (268–272)). In 1853, the pathologist Rudolf Virchow discovered that certain tissue deposits behaved in a similar manner to cellulose when stained by this technique and applied the term amyloid, meaning starch-like, to them (reviewed in (268–272)). Friedreich and Kekule were the first to recognize that the material found by Virchow in a waxy spleen, and believed to be amyloid, was most probably proteinaceous rather than cellulose-like (reviewed in (268–272)). Over a century later, in 1970, biochemical studies of isolated amyloid fibrils demonstrated that amyloid is indeed derived from protein (269) (see also section on historical perspective in amyloidosis).

Classification

The current amyloid nomenclature, which has been adopted by the World Health Organization and consistently recommended by the International Society of Amyloidosis Nomenclature Committee, is based on the chemical nature of the fibril protein (267,273). Thus, all amyloids begin with the designation A for amyloid, followed by a suffix that is an abbreviated form of the precursor protein's name (Table 22.1) (267,273). In primary amyloidosis, multiple myeloma–associated amyloidosis, and many cases of tumor-forming amyloidosis, where amyloid is derived from immunoglobulin light chains, the amyloid fibril is designated AL and the disease AL amyloidosis or AH if derived from immunoglobulin heavy chain.

The second major form of systemic amyloid, seen in amyloidosis secondary to chronic inflammatory conditions and autoinflammatory diseases such as familial Mediterranean fever (FMF), is derived from an acute-phase reactant, serum amyloid A protein (SAA); here, the amyloid type is designated AA. In addition, there is an expanding group of nonimmunoglobulin, non-AA amyloidoses. As of 2012, 30 types of precursor proteins have been associated with various clinical forms of the disease (267). There is an increasing number of heritable amyloidoses that are the consequence of a mutation in a precursor protein (Table 22.1). In these hereditary forms, in addition to the general amyloid designation derived from the name of the involved protein (e.g., ATTR = amyloidosis derived from transthyretin), the location of the mutation and the amino acid substitution are also indicated (please see further comments in sections on the corresponding amyloidoses). While some amyloidoses are localized, others are systemic or systemic and localized (267).

Amyloidosis is a rare disease. While the prevalence of amyloidosis in the general worldwide population is unknown,

TABLE 22.1 Systemic amyloidoses in humans: amyloid fibril proteins and their precursors

Amyloid protein	Precursor	Systemic (S) or localized (L)	Syndrome
AL/AH	Immunoglobulin light/heavy chain	S, L	Sporadic: primary, myeloma associated
AA	Serum AA protein	S, ?L	Sporadic: secondary, reactive; familial
ATTR	Transthyretin	S, ?L	Familial, sporadic—senile systemic
AFib	Fibrinogen A α-chain	S	Familial
AApoAI, II, IV	Apolipoprotein AI, AII, AIV	S, L	Familial, sporadic (aging)
AGel	Gelsolin	S	Familial
ALys	Lysozyme	S	Familial
ACys	Cystatin C	S	Familial
ALect2	Leukocyte chemotactic factor 2	S	Familial?
$A\beta_2M$	β_2-Microglobulin	S, ?L	Dialysis associated

according to estimates by the Mayo Clinic, its incidence is eight cases per million people per year in the United States (or 3000 people from the total population) (274). However, the disease may be underdiagnosed, both in the United States and worldwide. The prevalence of amyloidosis also increases with age. Among adult patients with nephrotic syndrome, approximately 1% to 5% have amyloidosis (275), but among the elderly (>85 years old), amyloidosis was the most common histologic diagnosis (16.9%) in a recently published large series (17,680) of native kidney biopsies from Spain (276). In contrast, amyloidosis is extremely rare in children where it has been diagnosed predominantly as AA amyloidosis, typically in association with autoinflammatory diseases or as hereditary amyloidosis (277–281). Among patients with AL, rare cases of patients <30 years of age have been identified (279,282). The autopsy incidence of systemic amyloidosis was 0.8% in one European series (283).

Prevalence varies depending on the population involved and the type of amyloidosis. The most common type in the United States and the Western world is amyloidosis associated with an underlying plasma cell dyscrasia (AL), followed by AA amyloidosis (284). A large series from the Mayo Clinic, examining data from 1977 to 1986, demonstrated that 74% of patients with amyloidosis were of the AL type, 4% were of the AA type, and, among the remaining patients, 20% had localized and 2% had familial amyloidoses (285). In a recently presented series of 445 patients with renal amyloidosis (284), immunoglobulin amyloidosis, primarily AL, accounted for 85% of cases, while AA, the second most common type of renal amyloidosis, was only three times more frequent than ALect2 (amyloidosis derived from leukocyte chemotactic factor 2) and six times more frequent than AFib (amyloidosis derived from fibrinogen).

This is in stark contrast to the situation in developing countries, where secondary (AA) amyloidosis is far more common than AL amyloidosis (286). In fact, worldwide, an estimated 45% of all systemic amyloidoses are of the AA type, which makes it the second most frequent type of systemic amyloidosis after AL (reviewed in (287)). Also, familial amyloidoses are diagnosed with increasing frequency, as awareness increases. While older series (285) showed that, among amyloid patients, only 2% had the familial form, more recent series (during 2000–2004) show that 10% of new patients (288) have genetic abnormalities.

The following sections describe the pathology of renal amyloidosis in general, followed by specific features of the various types of amyloidosis presented in the different sections.

Gross Pathology

Postmortem examination of patients with amyloidosis has generally revealed enlarged kidneys with pale, waxy-appearing cut surfaces. The first study of amyloidosis, by Bell, clearly demonstrated an increase in the weight of the kidneys, with only 5 of 65 cases showing either normal or small kidneys (87). In the study by Dikman et al. (289), which included 33 autopsies and 1 nephrectomy, from patients with different types of amyloidosis, the kidneys were over 200 g in 9 patients and between 100 and 200 g in 16 patients. By intravenous pyelogram, the kidneys were shown to be increased in size in 36 and smaller than normal in 5. Interestingly, the weight of the kidneys did not correlate with renal function or the site of renal amyloid deposition and was generally not proportional to the amount of renal amyloid (289). This indicates that, in advanced cases, parenchymal atrophy is the major factor governing kidney size. The average kidney weight of four patients with AL amyloidosis in a recent autopsy study from one of the authors was 200 g (82). However, it must be emphasized that amyloidosis, including renal amyloidosis, may not be grossly apparent.

Light Microscopy

The light microscopic features of amyloidosis are always the same, regardless of the type of amyloid. In hematoxylin and eosin–stained sections, amyloid appears as eosinophilic, amorphous, "hyaline" material (Fig. 22.18). In a PAS stain, amyloid is usually weakly positive. However, certain rare amyloid types (most notably AH) can be strongly PAS positive, but this is an exception rather than a rule (290).

Amyloid is defined by its affinity to Congo red and thioflavin T or S (Figs. 22.19 and 22.20) (267,291–293); the electron microscopic appearance of amyloid is also diagnostic (please see below). Although, in bright light, amyloid deposits, stained with Congo red, show a salmon pink or pale rose stain (Figs. 22.19A and 22.20A), this, in itself, is not diagnostic. A definitive diagnosis of amyloid requires examination under polarized light, with the demonstration of apple green birefringence (Figs. 22.19B and 22.20B). Conversely, small deposits of amyloid, which may not be apparent in bright light, can be

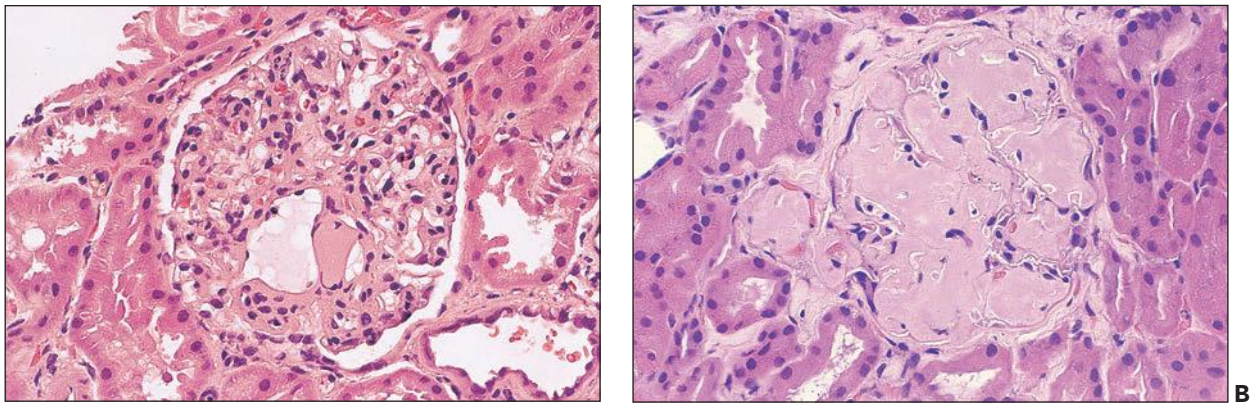

FIGURE 22.18 AL amyloidosis. A: Early segmental mesangial amyloid deposition. **B:** Complete obliteration of the glomerular architecture by amyloid deposits in an advanced case. (H&E, × 500.)

diagnosed through their green color when viewed under polarized light. In order to demonstrate small amounts of amyloid in tissue, thicker sections may be cut at 4 to 8 μm, instead of the customary 2 to 4 μm (294,295). In thicker sections, small deposits are less likely to be missed (as a consequence of unevenness of amyloid distribution within the examined tissue), and, therefore, sampling error is more easily avoided. However, the sections typically used in renal pathology (2 to 3 μm) are quite adequate for Congo red stain, providing the examination is performed properly. In order to minimize sampling error, the author (M.M.P.) routinely stains two slides with Congo red (and more if clinically indicated), preferably obtained from different levels within the block. Polarization microscopy is not trivial, or devoid of pitfalls, and adequate equipment is mandatory (295–297). Sensitivity of detection of birefringence depends on the intensity of the transmitted light (therefore, a strong light source is highly recommended) and pupil accommodation (hence, the examination of sections in a darkened room is also strongly recommended). Note, however, that if excess Congo red dye is retained by the tissue, it can lead to false birefringence (294). Note also that "preamyloidotic" deposits, that is, deposits that are positive for the amyloid precursor protein by immunohistochemistry, but negative by Congo red staining, have been detected in some

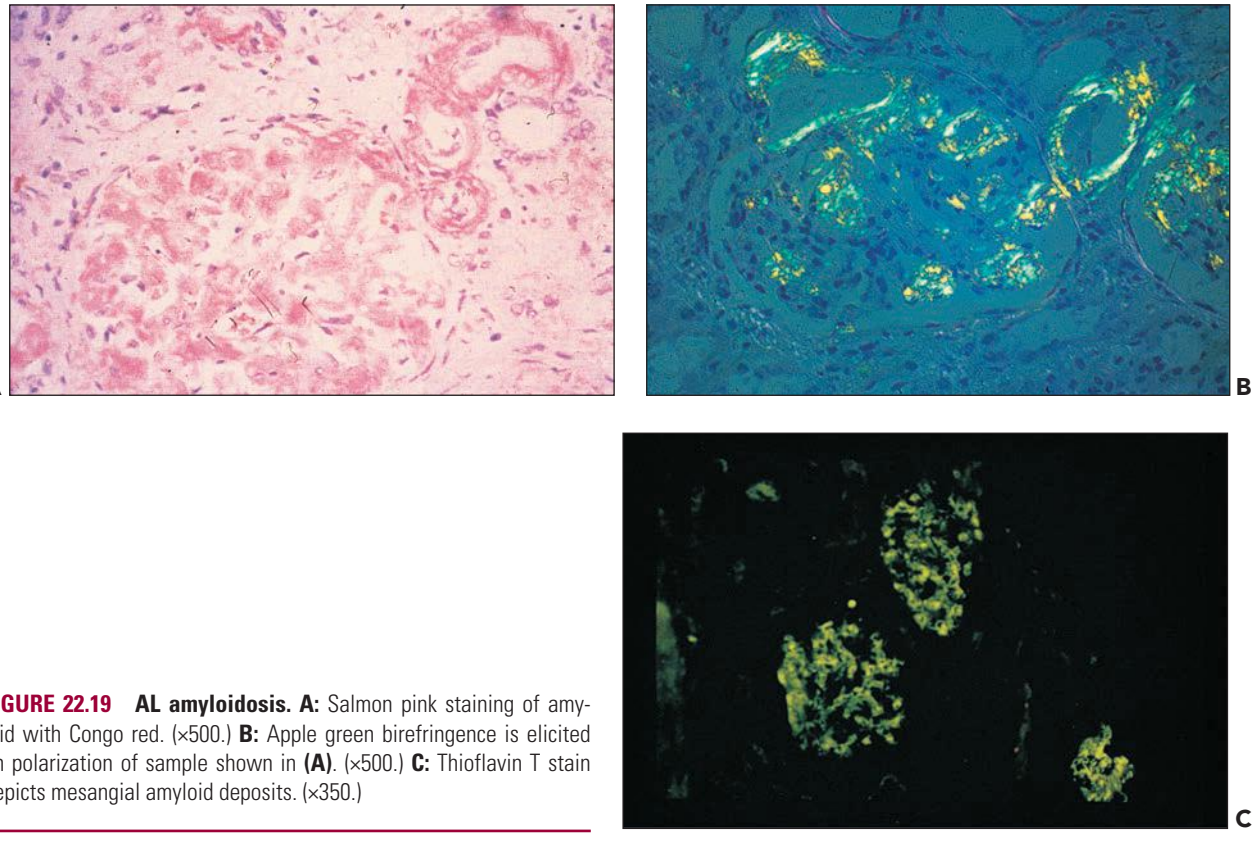

FIGURE 22.19 AL amyloidosis. A: Salmon pink staining of amyloid with Congo red. (×500.) **B:** Apple green birefringence is elicited on polarization of sample shown in **(A)**. (×500.) **C:** Thioflavin T stain depicts mesangial amyloid deposits. (×350.)

hereditary amyloidoses such as ATTR and dialysis-associated amyloidosis (298). In these cases, electron microscopy typically demonstrates nonfibrillar deposits. One important feature of polarization is "polarization shadow" where, at any given position of the specimen, only a portion of the amyloid deposit shows green birefringence (294,295). Only by rotation of the microscope stage will other birefringent parts of the section become visible while, in turn, the formerly visible areas will now be obscured by the "polarization shadow." Thus, as a consequence of this "polarization shadow" phenomenon, small deposits may be missed. This phenomenon can be avoided by use of additional methods such as fluorescent light and thioflavin stains (Fig. 22.19C). Interestingly, the Congo red dye itself is also a fluorochrome and can be examined under fluorescent light (294,295,299,300). Moreover, when Congo red–stained slides are examined by fluorescent light, the entire amyloid-containing area is highlighted (with no polarization shadow), which also facilitates the detection of small deposits (Fig. 22.20C). Different filters may be used in the Congo red fluorescence technique. With the fluorescein isothiocyanate (FITC) filter, deposits of amyloid appear orange, while, with a tetramethylrhodamine isothiocyanate (TRITC) filter, deposits are red. In the author's (M.M.P.) own experience, the latter filter gives a cleaner and more easily discernible visualization of amyloid.

A thioflavin stain, examined with fluorescence, is much more sensitive than Congo red/green birefringence or even Congo red/fluorescence and, therefore, is particularly useful in the detection of small deposits of amyloid (see Fig. 22.19C) (293). This stain, which is easy to perform, is particularly useful in renal pathology, where fluorescence microscopy is already used routinely. The greater sensitivity of the thioflavin stain is also augmented by the absence of the "polarization shadow" phenomenon. Thioflavin stains (T or S) have been used extensively in research. Other stains, such as crystal violet, methyl violet, Sirius red, and sulfated Alcian blue (SAB), are less sensitive and less specific. Thus, SAB stains glycosaminoglycans (GAGs) rather than the amyloid protein itself (295).

In the kidney, amyloid deposits can be found in any of the renal compartments: in the glomeruli, the interstitium, or the extraglomerular blood vessels. Glomerular amyloid formation begins in the mesangium (see Fig. 22.18), eventually replacing

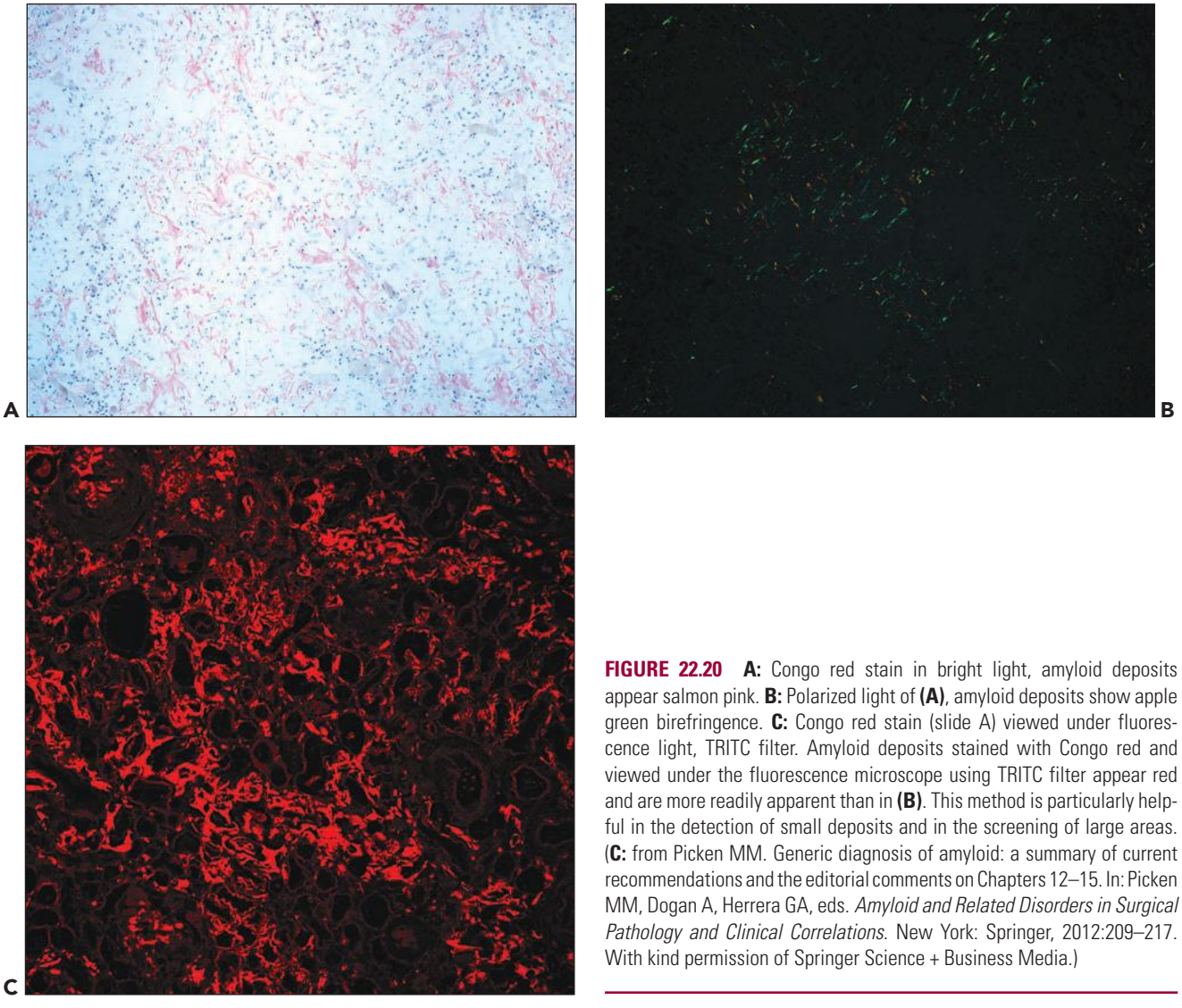

FIGURE 22.20 **A:** Congo red stain in bright light, amyloid deposits appear salmon pink. **B:** Polarized light of **(A)**, amyloid deposits show apple green birefringence. **C:** Congo red stain (slide A) viewed under fluorescence light, TRITC filter. Amyloid deposits stained with Congo red and viewed under the fluorescence microscope using TRITC filter appear red and are more readily apparent than in **(B)**. This method is particularly helpful in the detection of small deposits and in the screening of large areas. (**C:** from Picken MM. Generic diagnosis of amyloid: a summary of current recommendations and the editorial comments on Chapters 12–15. In: Picken MM, Dogan A, Herrera GA, eds. *Amyloid and Related Disorders in Surgical Pathology and Clinical Correlations*. New York: Springer, 2012:209–217. With kind permission of Springer Science + Business Media.)

the normal mesangial matrix and extending into peripheral capillary walls. Amyloid deposition may occur in segmental, diffuse mesangial, nodular, and pure basement membrane patterns (275,289,300–303). Early segmental amyloid deposits are small, discrete, and confined to the mesangium and, hence, may be unsuspected on H&E-stained sections. It is very easy to miss this early form of amyloidosis, and the careful evaluation of special stains, immunofluorescence, and electron microscopy is required for a correct diagnosis. It is also recommended to examine a Congo red stain in order to exclude the presence of early amyloid deposits in particular in patients with proteinuria/nephrotic syndrome not associated with immune complexes or in unexplained renal failure. In the diffuse form, the mesangium is uniformly expanded by weakly PAS-positive acellular deposits. In the case of amyloid derived from fibrinogen (AFib), near complete glomerular obliteration by amyloid is typically seen (303–305). In the nodular form, the mesangium is asymmetrically expanded by masses of amyloid that may compress and compromise the capillary spaces (110,301,306). The nodular form should be distinguished from diabetic nephropathy and other forms of nodular glomerulosclerosis. The glomerular lesions may be uniform among the lobules within a glomerulus. The basement membrane may remain normal even when massive mesangial amyloidosis is present. However, fraying, loss of argyrophilia, and discontinuities are seen along the glomerular capillary walls with the Jones methenamine silver stain as amyloid deposits increase in this location. A multinucleated giant cell reaction may accompany glomerular amyloid deposition (307). Rarely, crescents can be seen, highlighting the fact that capillary wall rupture has occurred (308). Subepithelial amyloid deposition may be associated with the formation of irregular basement membrane "spicules" due to the presence of amyloid deposits arranged in parallel arrays perpendicular to podocytes. This is associated with the loss of argyrophilia and fraying of the outer aspect of the basement membrane. Similar changes can also be seen at the periphery of mesangial areas (please see also below under Electron Microscopy) (87,289,309).

Exclusively glomerular involvement may be seen in some patients and in some types of amyloid, typically in AFib, but recently also in AL (305,310,311). The tubulointerstitial compartment may be variably affected. Interstitial and peritubular deposits of amyloid are seen in approximately 50% of cases (307), with amyloid deposition beginning generally in areas adjacent to blood vessels. Medullary amyloid deposits are more frequent and more extensive, with a predilection for deposition around the vasa recta, loops of Henle, and collecting ducts (289,307). Watanabe and Saniter (307) reported interstitial involvement in 57 of 122 cases of amyloidosis, and in 12 cases, it was characterized as massive. Occasionally patients with AA amyloidosis and amyloidosis derived from certain mutants of transthyretin and apolipoprotein AI and AIV may show amyloid deposition limited to the interstitium and medulla (312,313). Such patients present with renal failure not associated with proteinuria (303,314–316). Inflammation is not a typical feature of amyloidosis. However, while scattered aggregates of lymphoplasmacytic cells may be present, in particular in AL (289), even more inflammation can be seen in patients with AA amyloidosis derived from "skin popping" (V. D'Agati, personal communication). Aggregates of foam cells can also be seen in the interstitium, primarily in cases with large amounts of proteinuria (289). The tubules may show nonspecific findings or vacuolization and damage at the early stages, while tubular atrophy is seen most often in advanced cases with interstitial amyloid deposition.

Another site of significant amyloid deposition is the renal vasculature. Among renal vessels, arteriolar deposits are most frequent, followed by arteries, peritubular capillaries, and veins (289). The amyloid deposits in the vessel walls may be subtle or may completely replace the vessel walls and be associated with occlusion of the lumina. Vascular amyloid may be mimicked by hyalinosis and even fibrinoid necrosis, and special stains are recommended to establish a distinction. Vascular deposits frequently coexist with glomerular amyloid, but the extent of vascular amyloid deposition may be unrelated to the pattern of glomerular involvement. Watanabe and Saniter found amyloid in interlobular arteries or arterioles in 97 of 122 renal biopsies (307). In rare cases, the vessels are the only site in the kidney where amyloid can be demonstrated. In some familial amyloidoses, for example, AApo AII, amyloid deposits may involve the blood vessels and spare the glomeruli (303). In certain cases of AA, and even in rare cases of AL amyloidosis, only vascular deposits were detectable in the kidney (77,317–319).

In many suspected cases of amyloidosis, an abdominal fat biopsy may be diagnostic. The latter is as sensitive as rectal biopsy and more so than bone marrow biopsy. The sensitivity (52% to 100%) and the specificity (99%) suggest that, although a positive result is good evidence for the disease, a negative result does not exclude it (295,320,321). An adequate amount of tissue is mandatory, and therefore, a small surgical biopsy, or a deep skin punch biopsy, may be preferred to a fine needle aspiration biopsy (295). A simple but effective scoring system for estimating the amount of amyloid in a fat biopsy sample is currently in use in many laboratories and has been recommended for reporting (320,322).

Amyloid Typing

In view of the recent emergence of amyloid type–specific therapies, precise identification of the amyloid protein type (hereinafter referred to as "amyloid typing") has now become the standard of care (Figs. 22.21 and 22.22). While traditionally kidney biopsies have been analyzed by frozen section immunofluorescence, in the case of amyloid, other methods have also been applied to amyloid typing. To this end, various antibody-based techniques, immunofluorescence stains on frozen tissues, immunoperoxidase stains on paraffin sections, immunoelectron microscopic labeling procedures, and Western blot methods have been used with varying levels of success (156,323–329). The typing of amyloid proteins in formalin-fixed paraffin-embedded specimens by biochemical methods has also been reported (330–332). This latter approach was advanced markedly through the availability of more sophisticated proteomics methods (332–334) (please see also Section Proteomics). While the more complex and newer techniques are available only in specialized laboratories, immunofluorescence on frozen section and immunohistochemistry on paraffin sections still are widely used in clinical practice (335,336). However, it must be stressed that the immunohistochemistry of amyloid differs significantly from that encountered in other areas of general surgical pathology and caution as well as experience are necessary for its interpretation.

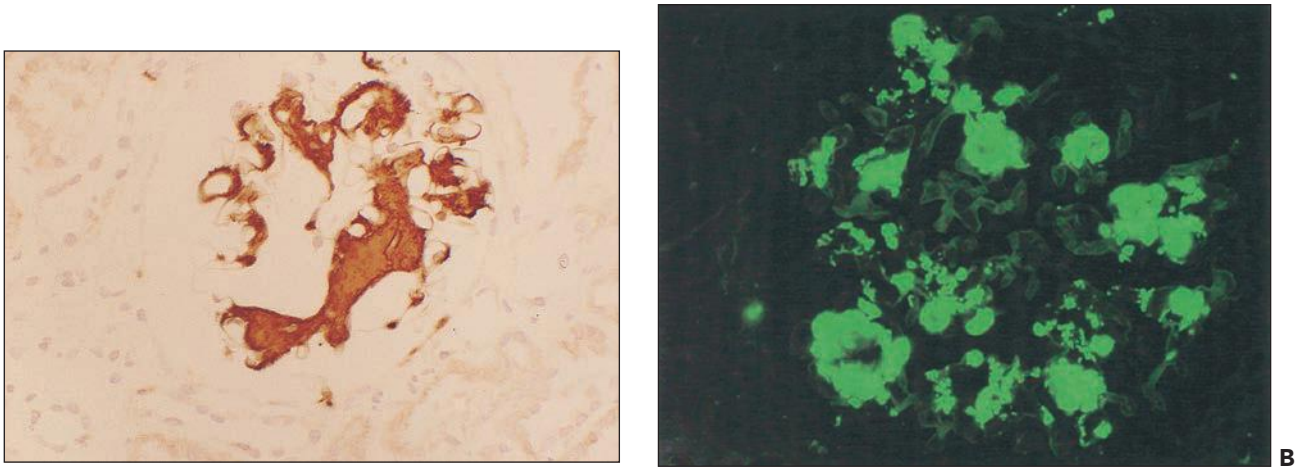

FIGURE 22.21 **A:** AA amyloidosis. Distinct mesangial staining for AA protein. Immunoperoxidase stain of a paraffin section for AA protein. (×500.) **B:** AL amyloidosis. Mesangial deposits of λ chain amyloid are clearly depicted; stain for κ light chain was negative (not shown). Direct immunofluorescence for λ light chain. (Fluorescein, ×350.)

Amyloid Immunohistochemistry

Amyloid immunohistochemistry presents the pathologist with a serious challenge because of (a) the lack of specific antibodies for the amyloid proteins (as opposed to the native proteins), (b) the heterogeneity of amyloid proteins (further compounded by protein variants) and the lack of specific antibodies for all different amyloid types, and (c) the high background staining that accrues from serum contamination competing with the signal from the amyloid protein (324,325).

Amyloid type–specific antibodies are limited in their availability, and regulatory issues (validation of such antibodies as analyte-specific reagents [ASR]) have not been uniformly addressed. Commercially available antibodies are typically raised against epitopes of the "native" protein, which may be altered in the corresponding amyloid fibril protein due to conformational changes and/or the fragmentation/truncation that occurs during fibrillogenesis. This is particularly true in AL, where the fibril may be derived predominantly from a fragment of the light chain or a mixture of fragments of various lengths. Since commercial antibodies are typically raised against the constant region, antibody reactivity depends on the extent to which this region is present in the amyloid fibrils. In one large study, in 31% of AL patients, immunohistochemistry on paraffin sections using commercial antibodies was "not entirely" diagnostic (326). In two other studies reported by large amyloidosis referral centers, immunohistochemistry on paraffin sections was diagnostic in 92% (utilizing a combination of commercially available as well as custom antibodies) (327) and in 97.8% (utilizing custom antibody-specific antibodies) (325). Amyloid derived from the heavy chain (AH) has been only very rarely reported (290,337–342). In a few earlier reported cases of AH, which were studied by biochemical methods, the involved heavy chains, either γ or μ, had deletions in the C_H1 and C_H2 regions (337–342). Hence, it is suspected that in some cases commercial antibody reactivity may be diminished or abolished altogether. In some cases of hereditary amyloidoses, antibody reactivity is dependent on the type of mutation (324). However, reactivity of various antibodies with amyloid fibril proteins appears to be also, to some extent, a technique-dependent phenomenon. Thus, it has been shown that antibody typing by immunoelectron microscopy and immunoblotting (Western blot) both give better results than typing in paraffin sections (157,328,329). While further consideration of these issues lies outside the scope of this chapter, they indicate that there are many aspects of amyloid fibril protein structure and composition that are still only partially understood. Antibody reactivity may also be affected by the presence of other components, such as amyloid P component, GAGs, variable extracellular components, and even lipids, which are also present in amyloid deposits.

In immunohistochemistry, each diagnostic stain should be accompanied by a positive and negative control stain. However, with amyloid not being a single entity, such approach is not practical. To circumvent this issue, a surrogate positive control stain and a comparative immunohistochemistry have been used. Amyloid P component is present in all types of amyloid and hence can be used as a "universal" positive control and a surrogate for amyloid control positive stain (343). It also shows good reactivity in both paraffin and frozen sections (324). Comparative immunohistochemistry is based on interpretation of a panel of antibodies rather than a single antibody. This facilitates a distinction between a true positive stain from nonspecific background stain. The strongest stain, comparable in intensity to stain for amyloid P component, is interpreted as diagnostic (324,325) (Fig. 22.22). Using a single antibody, rather than a panel, or using different methods such as immunofluorescence for some and immunoperoxidase for other antibodies is very risky since it does not allow a comparison of intensities of stains obtained by different techniques. Contaminating serum proteins can compete with the signal from the amyloid protein itself and create a high background stain. This issue is particularly difficult to resolve in paraffin sections. In contrast, in frozen sections, plasma proteins are largely eliminated simply by washing (324,344). To some extent, however, even in vivo, various serum proteins can be adsorbed to amyloid deposits. Immunohistochemistry results must be interpreted in the context of Congo red stain. The combination of a traditional Congo red stain with

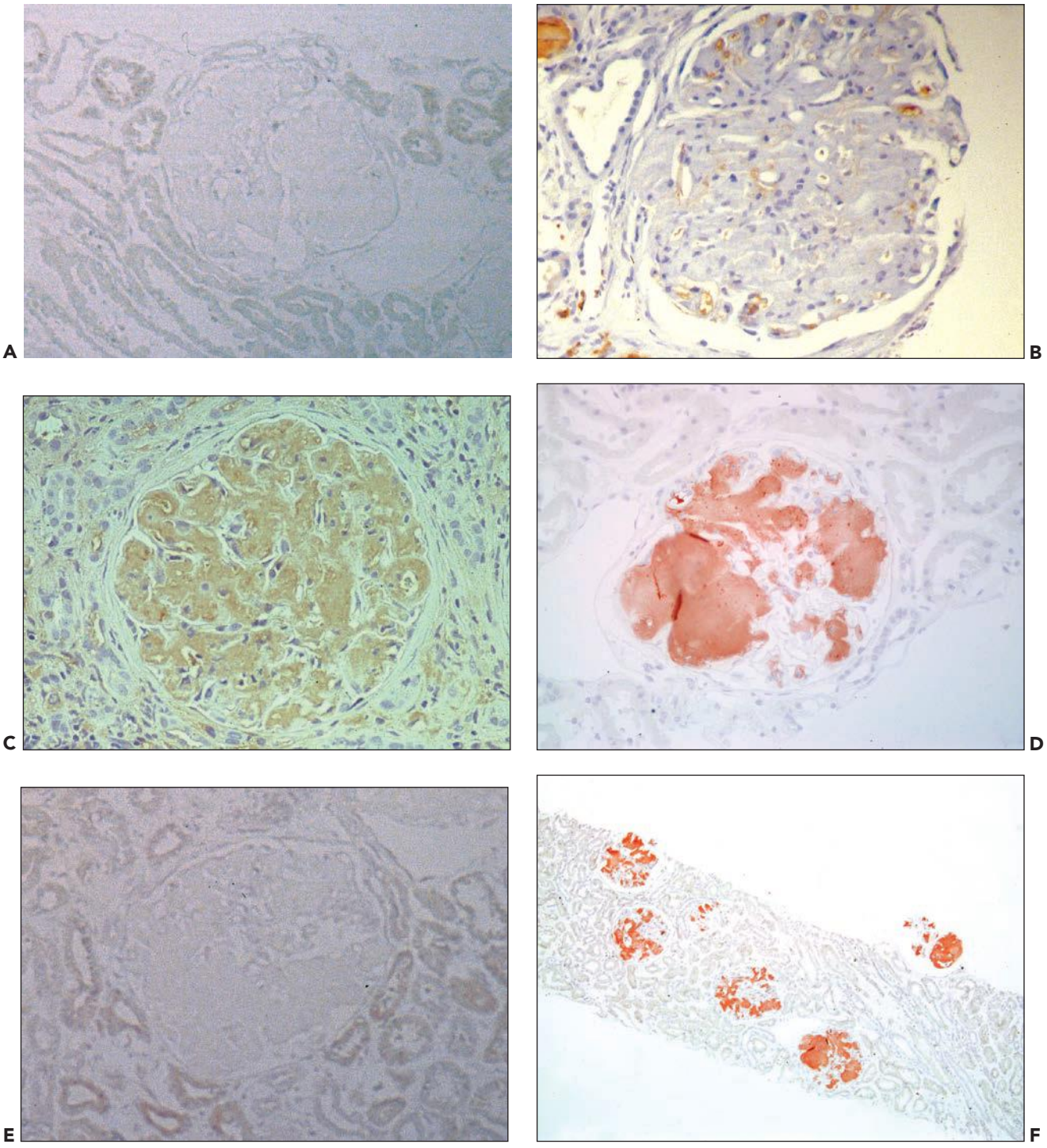

FIGURE 22.22 Kidney with AFib. Amyloid immunohistochemistry in paraffin sections demonstrating advantages of using a panel of stains. **A:** Negative stain for amyloid A protein. **B:** Deposits of amyloid are negative for λ light chain; positivity for this antibody is seen focally in the lumen of glomerular vessels. **C:** Stain for κ light chain showing weak (1+) positivity of amyloid deposits. **D:** Stain for amyloid P component showing strong positivity (3+). **E:** Negative stain for transthyretin. **F:** Amyloid deposits are strongly immunoreactive for fibrinogen (3+) and are limited to the glomeruli. All paraffin sections, immunoperoxidase stains; no counterstain **(A, E)**, all other with hematoxylin counterstain. Magnifications: ×280 in **(A)** through **(E)**; ×60 in **(F)**. (From Picken MM, Linke RP. Nephrotic syndrome due to an amyloidogenic mutation in fibrinogen A alpha chain. *J Am Soc Nephrol* 2009;20:1681.)

immunohistochemistry—the "overlay technique"—has also been described (325,326).

In general, the main differential diagnosis of the amyloid type is AL versus other types (280,284,327,335,336,345–348). In the kidney, >90% of amyloids are of the AL-AA types, with other types being individually rare but collectively significant, approaching 10%. In contrast, in cardiac and peripheral nerve biopsies, amyloid derived from transthyretin, ATTR, is the second most common type of amyloid after AL (348). In the kidney, the typical initial antibody panel should include antibodies against kappa and lambda immunoglobulin light chains, amyloid A protein, transthyretin, fibrinogen, and antibody against human AP component. A stain for β_2-microglobulin should be added in the setting of dialysis (324).

Note that a single patient may suffer from different amyloid diseases simultaneously. Examples include renal glomerular AL and vascular AA deposits, AL and AA deposits in different biopsy sites or the same biopsy site but involving different structures (mucosa and submucosal vessels respectively), and cardiac amyloid derived from wild-type ATTR and AApo AIV, both in anatomically distinct patterns of distribution (317,325,326,349). The significance of rare reports of more than one amyloid protein type being demonstrable seemingly in the same amyloid deposits is at present unclear (290,350–354). In the author's own experience (M.M.P.), enhanced positivity for AA amyloid protein was seen in a patient with a long history of Crohn diseases who developed AL-κ systemic amyloidosis (354). It is feasible that the existence of amyloid deposits may induce polymerization of another potentially amyloidogenic (amyloidogenesis-prone) protein. It is also becoming evident that immunohistochemical stains can detect deposits of amyloid protein that are Congo red–negative and, hence, considered to be preamyloidotic. This has been documented in occasional patients with hereditary amyloidosis (ATTR) and dialysis-related amyloidosis (298).

In general, paraffin section immunohistochemistry is particularly challenging for diagnosis of AL/AH; similar considerations also apply in the case of deposits of ATTR (amyloid derived from transthyretin) and certain cases of other hereditary amyloidoses (324–327) (see Figs. 22.21A and 22.22). Antigen retrieval methods can be tricky (326).

Immunofluorescence

In the authors' own experience, amyloid typing using immunofluorescence on frozen sections is superior to immunohistochemistry on paraffin sections where the issues of altered antibody reactivity and background staining are less of an impediment to successful amyloid typing (324,355). In general, most antibodies perform better in frozen than in paraffin sections, and this is especially evident in the detection of serum proteins in kidney biopsies, as well as the detection of light chain restriction, in general, and AL, in particular (see Fig. 22.21B) (324,355–357). The proportion of renal biopsies with subsequently proven AL, where the amyloid deposits were not reactive in frozen sections with antibodies against kappa or lambda light chain, varies between 13.6% (M.P., author's own experience) and 35.3% (346,358–361). On rare occasion, both light and heavy chain immunoglobulins have been detected by immunofluorescence or mass spectrometry in amyloid deposits (290,362,363). While such reports are intriguing, additional studies are needed to establish whether such a combination truly represents a distinct amyloid type or an artifact (364). In a recently described large series of immunoglobulin renal amyloidosis, 7.38% of cases seemingly showed either heavy chain or a mixture of light and heavy chains (290).

While in the majority of cases, immunofluorescence stains performed on frozen sections demonstrate clear light chain restriction, verification of such result against a short panel of stains including AP is prudent (324). Since, currently, in most laboratories, stains for kappa and lambda light chains, gamma, alpha, and mu heavy chains and fibrinogen are routinely included in the immunofluorescence antibody panel of all native kidney biopsies, in most instances, amyloid typing would require only additional stains for AP component, AA, and transthyretin; β_2-microglobulin stain should be added for patients on dialysis. Testing for light chains should be done routinely on all native kidney biopsies, at least on those from adult patients. This not only will allow the successful typing of many cases of AL amyloid but is also critical in the detection of other kidney pathologies associated with an underlying plasma cell dyscrasia/B-cell lymphoproliferative disorder (see earlier in this chapter 956–976).

Regardless of the method used (frozen or paraffin sections), it is critical that very stringent criteria are applied to the interpretation of results, and all cases with equivocal results are best delegated to a specialized reference laboratory (324–328). Lachman et al. (365) studied 350 patients with systemic amyloidosis, in whom the diagnosis of AL was suggested by clinical and laboratory findings and by the absence of a family history. This study showed that 9.7% of patients actually had familial amyloidosis. Interestingly, a low-grade monoclonal gammopathy was detected in 24% of these patients. Thus, both careful clinicopathologic correlation as well as the *exclusion* of other amyloid types are important.

Proteomics

Various nonantibody-based analytical tools, derived from proteomics, have been tried in the typing of amyloid deposits. Apart from the avoidance of issues related to reduced sensitivity and specificity that may be encountered in immunohistochemistry, the goal has been to develop methods that are independent of antibodies or a predetermined knowledge of the patient's amyloid type; this not only would permit a reliable diagnosis of the amyloid type but may also lead to the discovery of previously unknown types of amyloid. The aim was to develop techniques that would be applicable to the small biopsies that are typical among clinical specimens. The emergence of proteomics methods has facilitated the use of smaller samples and, ultimately, also, paraffin-embedded tissues (332–334).

The rationale for the application of proteomics methods to amyloid typing lies in the relative abundance of amyloid protein in the tissue, where it is frequently the dominant protein. Although the initial approach was to study the entire proteome (shot gun proteomics), over the years, the techniques have been refined to include sample enrichment methods, such as laser microdissection, and the identification of specific protein targets (targeted proteomics), in order to increase specificity. While the details are beyond the scope of this book, the salient features are summarized below.

Briefly, in order to increase the amyloid content in the analyzed sample, Congo red–positive areas are excised using laser microdissection and captured. In such enriched tissue

samples, almost pure amyloid deposits are present with minimal background tissue. The proteins contained within the collected tissue samples are extracted and subsequently subjected to enzymatic digestion by trypsin yielding small peptides suitable for processing by liquid chromatography (LC) and tandem mass spectrometry (MS/MS). Each peptide present in the human proteome has a unique fragmentation pattern. Therefore, using computer algorithms, it is possible to predict the amino acid sequence of the peptide analyzed by MS/MS. The amino acid sequences are deduced from a comparison of the observed fragmentation pattern of each peptide with the theoretical fragmentation pattern of all human tryptic peptides predicted by the human genome. A probability score is calculated for individual peptides and ultimately for a given protein. To increase the specificity of the method, multiple peptides from a given protein are analyzed. Although this process is not based on true sequencing of the protein, but rather on complex computation of various algorithms, the currently used MS/MS methodology is deemed to be highly accurate and reproducible, to the extent that it may be used with confidence in clinical applications (332–334,353). Given the challenges encountered with amyloid typing in paraffin sections, this is indeed a welcome development. However, like all laboratory methods, no single method is free from limitations. For MS/MS, these include the following:

1. Identification of the amyloid protein is based on the premise that this protein is the most abundant species within the analyzed sample. However, other proteins may be present within amyloid deposits, such as amyloid P component, apolipoprotein E, and various serum proteins.
2. Conversely, small amyloid deposits may be associated with low-abundance proteins/peptides in the examined tissue and, thus, may be obscured by more abundant proteins.
3. The observed peptide fragmentation data must be matched to *known* protein sequences that are available in public databases, and hence, certain germ-line polymorphisms or somatic mutations may not be identified.
4. The protein under study must contain areas amenable to digestion by trypsin in order to generate peptide fragments of appropriate size for MS, which are necessary for the protein to be identified. To this end, Rowczenio et al. (366) recently reported one patient where mass spectrometry–based proteomics technology could not be used due to the presence of numerous trypsin cutting sites in an area of the protein that could not be detected by MS.

These limitations notwithstanding, it has been possible to identify virtually all known amyloid protein types, and thus, the number of cases with undetermined amyloid type has been markedly reduced to 7% with 3% of samples being deemed to be insufficient for analysis (367).

Comparison of Mass Spectrometry With Antibody-Based Amyloid Typing

MS technology has been very helpful in achieving the unambiguous amyloid typing of formalin-fixed paraffin-embedded tissues. It has also been critical for the typing of cases with limited antibody reactivity and in the discovery of new protein types and identification of their variants. Additional area where MS data may be helpful includes the delineation of the spectrum of immunoglobulin-derived amyloid. To this end, published MS data indicate that in most cases of AL, the involved light chain contains both variable *and* constant regions, the latter being present at least in part (334). While earlier studies emphasized the presence of the V region in AL, several recent reports have also highlighted the hitherto underestimated role of the C region in amyloid fibril formation. Thus, it has now been demonstrated that the C region may be involved in initiating aggregation and providing a template for V region deposition (368). Recently, detection of both heavy and light chain components has been reported by both immunofluorescence and MS (290,360,362). However, additional studies are needed in order to establish that this truly represent a new entity as has been done for other proposed new entities (360,369).

However, although MS technology is gaining in popularity in the institutions having access to it, the question remains whether the technique should replace, or complement, the existing methods. Currently, the latter approach would seem to be a safer and more reasonable choice. In this connection, the discovery of new amyloid types by MS is being validated with immunohistochemistry. Also, at the present time, antibody-based methods would seem to be better suited to the detection of very small deposits, while the effectiveness of the MS technique is currently limited by the abundance of amyloid in the tissue examined (366,370). Now that emphasis has shifted decidedly toward the early detection of amyloid in patient specimens, where very small deposits are expected rather than the exception, this may be to the relative disadvantage of MS. Other limitations of each of the detection methods are discussed above.

Recommendations on Reporting

An amyloidosis-related pathology report should include the following important points: (a) the method used for amyloid detection, (b) the method(s) used for amyloid typing, and (c) the pattern of distribution of amyloid in the patient specimen (322). For antibody-based testing, the intensity of each stain (in relation to amyloid distribution) should be reported; the results of additional studies, if performed/known, should also be included. A scoring system for amyloid involvement of the kidney has been proposed, but, thus far, it has been neither validated nor widely accepted. This is due, in part, to its complexity (302,371). This issue is currently under discussion (322).

Electron Microscopy

Regardless of amyloid type, the ultrastructural findings are essentially similar: Amyloid is characterized by randomly disposed, rigid, nonbranching, variably long (up to 1600 nm), 7- to 10-nm-diameter fibrils (110,372–376) (Fig. 22.23). There are reports suggesting that β_2-microglobulin–associated amyloidosis has some unique ultrastructural characteristics (377–381). The authors noted that the amyloid fibrils in this condition exhibited a peculiar arrangement, forming short curvilinear rather than straight bundles (369,377–381). At high resolution, exceeding that used in diagnostic pathology, amyloid fibrils consist of fibrillary subunits, termed "protofilaments." In mature fibrils, protofilaments can vary in number and can twist around each other forming a hollow fibril core (371,382).

Amyloid deposits may be found in any of the renal compartments, as shown by ultrastructural immunogold labeling (see Figs. 22.18 and 22.23). In glomeruli, fibrils are invariably

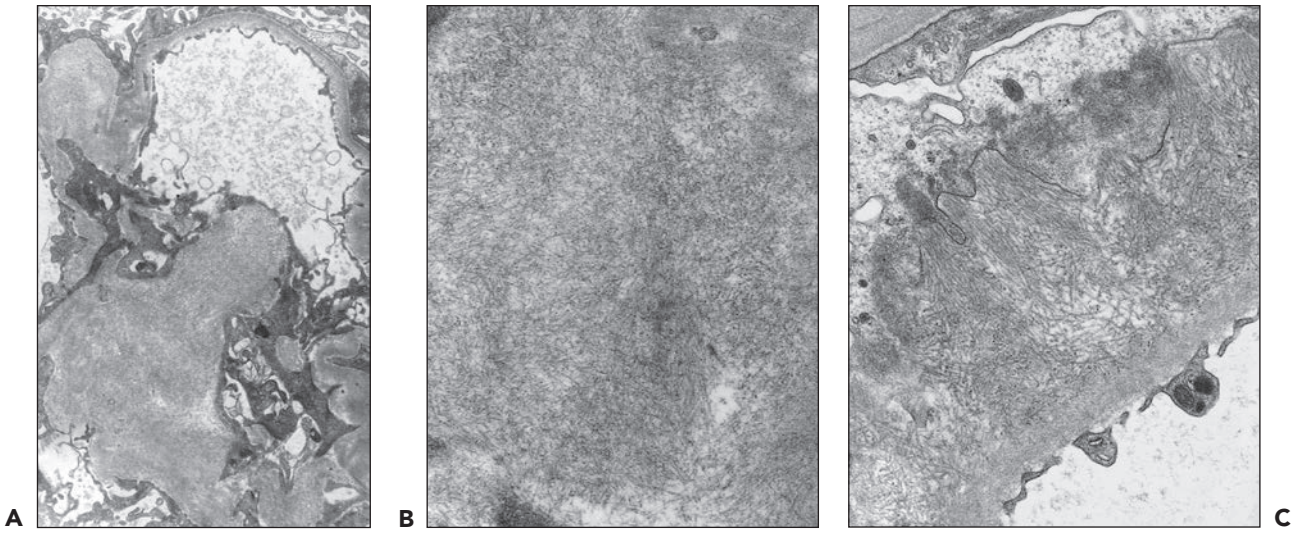

FIGURE 22.23 AL amyloidosis. Typical ultrastructural appearance of amyloid fibrils seen in the mesangium **(A and B)** and extending into the peripheral glomerular capillary wall and occupying a subepithelial location **(C)**. Transmission electron microscopy. (Uranyl acetate and lead citrate.) (**A:** ×7500; **B:** ×12,500; **C:** ×13,500.)

found in mesangial areas, replacing the normal mesangial matrix. They may also extend into the peripheral capillary walls, occupying the subendothelial or subepithelial spaces (375). Along the latter, fibrils may be arranged in parallel arrays perpendicular to the podocytes forming feathery "spikes" (see Fig. 22.23C) (371,380). Infiltration of the basement membrane by amyloid may be associated with the loss of argyrophilia and the fraying seen by light microscopy. New layers of the basement membrane may be seen surrounding amyloid deposits. In end-stage amyloidosis, the entire lamina densa of the glomerular basement membrane may be replaced by amyloid, and amyloid may be found in the urinary space and the Bowman capsule. Extremely rare cases of amyloidosis may exhibit massive aggregates of amyloid fibrils in subendothelial and mesangial areas, arranged in tightly packed electron-dense structures, which can be confused with other entities, including membranoproliferative glomerulonephritis, cryoglobulinemic glomerulopathy, and even diffuse proliferative lupus nephritis (374,376,381). Such cases show the expected tinctorial characteristics of amyloid and are typically associated with monoclonal κ light chains. In the few documented cases of regression of amyloidosis, the glomerular basement membrane remains as a complicated lattice of basal lamina, and there may be a few remaining fibrils or amorphous debris in between the lacunae left behind by the lamellated lamina densa (375). The resulting overall appearance mimics that of stage IV membranous nephropathy.

Amyloid is defined as "mainly" an *extracellular* accumulation of fibrils sharing similar staining and ultrastructural features (267). However, the concept of intracellular fibrillogenesis also exists. Various intracellular structures, some with complete (neurofibrillary tangles) and others with partial (inclusion bodies) properties of amyloid, have been encountered in various degenerative diseases (267). Small intracellular aggregates with amyloid properties have been demonstrated in the choroid plexus, in the adrenal cortex, and in the Sertoli cells (383). Early electron microscopic studies also suggested intralysosomal formation of amyloid fibrils (384). Fibrillary amyloid-like structure of crystalline inclusions was also observed in rare cases of light chain proximal tubulopathy (385). Two additional cases of light chain proximal tubulopathy with intracellular lysosomal-like structures exhibiting Congo red positivity and green birefringence were also recently reported (104,386). It is quite possible that in the future, the pathologic definition of amyloid will be extended to include intracellular structures as well. It should be added that the molecular definition of amyloid differs from that used in pathologic diagnosis. At the molecular level, amyloid is defined on the basis of the characteristic conformational arrangement of the proteins, consisting of highly ordered cross-beta sheet structures. This definition therefore extends to synthetic peptides and proteins forming amyloid in vitro.

AL/AH and AA Amyloidosis

Historical Perspective

The first case of amyloidosis associated with myeloma (discovered retrospectively, since myeloma was not yet recognized as a pathologic entity) was reported in 1867 (387). In 1872, Adams documented an association between renal amyloidosis and myeloma (387). The first case of amyloidosis in a patient with myeloma was published in 1902 by Jochmann and Schumm (388), and 15 years later, Glaus (389) reported a second case of amyloidosis associated with myeloma. Approximately 50 years later, Schmiedeberg described the amino acid composition of amyloid and noted that it resembled that of serum globulin (270). The Congo red stain was introduced in 1933 by Bennhold as a diagnostic clinical test and later used as a histologic stain (390,391). Six years later, Divry and Florkin reported green birefringence when amyloid was stained with Congo red and then viewed under polarized light (392). In 1931, Magnus-Levy first suggested a relationship between amyloid and light chain proteinuria (393). Two years later, Bell studied 90 autopsies from patients with multiple myeloma (including a literature review) and discovered 3 patients with amyloidosis, further substantiating a possible association (87).

In 1949, Sikl, in another literature review, compiled information from 40 cases with amyloidosis and myeloma and clearly demonstrated that the two diseases were not merely coincidental (394). It took almost another 30 years (in 1959) for Cohen and Calkins (395) to demonstrate the fibrillary nature of amyloid using the transmission electron microscope. In 1961, Osserman recognized that abnormal light chains were directly involved in the pathogenesis of amyloidosis (396). Four years later, Eanes and Glenner reported for the first time the fact that amyloid was composed of β-pleated sheets (397), and in the early 1970s, Glenner et al. (398) further demonstrated the amino acid composition of an amyloid protein, which matched the N terminus of an immunoglobulin light chain. In 1972, Levin et al. (399) described the amino acid composition and sequence of amyloid from a patient with secondary amyloidosis and labeled it *amyloid A protein*. In the late 1960s and early 1970s, Shirahama and Cohen emphasized the essential role that mesangial cells played in the pathogenesis of glomerular AA amyloidosis (384,400). Their studies with splenic macrophages engaged in amyloid formation also strongly suggested a crucial role for lysosomes in the process of amyloidogenesis. Linke et al. in 1973 (401) and Epstein the following year (402) digested BJ proteins with proteolytic enzymes, forming amyloid fibrils.

The study of renal amyloidosis was then essentially ignored until the early 1990s, when the work of Solomon et al. (60), who injected light chain proteins intraperitoneally, demonstrated the formation of vascular amyloidosis in the kidney. This seminal work was followed by elegant studies on the molecular modeling of amyloid and analysis of the amino acid sequence and conformation of amyloidogenic light chains. In 1996, Tagouri et al. (403) demonstrated amyloid formation by mesangial cells incubated with amyloidogenic light chains purified from the urine of patients with AL amyloidosis, thus establishing an in vitro model to study renal amyloidogenesis. This was followed by the use of an isolated kidney perfusion model, whereby amyloidogenic light chains were delivered to the kidney via the renal artery (404). Additional studies by Comenzo et al. (405) convincingly showed that λ VI light chains display a striking renal tropism and, as a consequence, are the most common light chains involved in renal amyloidosis, as had been previously proposed by Solomon et al. (54). An in vivo model of renal amyloidogenesis followed. The first case of heavy chain–related amyloidosis was reported by Eulitz et al. in 1990 (337), and since then, only relatively few of similar patients have been documented (338–342).

AL/AH Amyloidosis: Clinical Presentation and Laboratory Findings

The clinical manifestations of AL/AH amyloidosis in general are frequently rather nonspecific and virtually always include fatigue and weight loss. Other findings may include edema, orthostatic hypotension, hepatomegaly without filling defects, peripheral neuropathy, cardiac arrhythmias, carpal tunnel syndrome, and congestive heart failure resulting from restrictive cardiomyopathy (406). Gastrointestinal symptoms (motility abnormalities, atony, pseudoobstruction, malabsorption, diarrhea, bleeding) may also be seen in fewer than 10% of patients. In 8% of patients with AL, gastrointestinal symptoms are the dominant syndrome (406). Hepatic involvement is more common, involving some 25% of patients with AL amyloidosis (406). The most common clinical presentation is proteinuria with or without renal insufficiency (406). The amount of proteinuria is very variable and depends on the extent of renal involvement. Renal failure may ensue in the advanced stages.

The kidneys may be involved in all types of amyloidosis. Glomerular involvement by amyloidosis is frequent and explains the proteinuria that these patients exhibit. Amyloidosis is often diagnosed by renal biopsy. Only a small percentage of patients have an established diagnosis of amyloidosis prior to renal biopsy.

Amyloidosis related to plasma cell dyscrasia is also called *primary amyloidosis*. In most cases of immunoglobulin-associated amyloidosis, the precursor protein is an immunoglobulin light chain or fragment (AL amyloidosis). In a minority of the cases, amyloidosis originates from truncated immunoglobulin heavy chains (AH amyloidosis) (337–342). In these cases, there is an underlying plasma cell dyscrasia responsible for the amyloidosis.

AL/AH amyloidosis primarily affects older individuals; it is unusual to find a patient with this condition younger than 40 years of age. Early studies of renal amyloidosis by Bell clearly documented that all renal compartments may be involved and that it was a systemic disease; in only 2 of the 65 cases reported, amyloid was found only in the kidney (87). However, renal involvement may be the first manifestation of systemic disease, with subsequent studies demonstrating the systemic nature of the amyloidosis. Extrarenal localized AL amyloid deposits are frequently seen in the genitourinary tract (see under Localized Amyloidoses).

Etiology and Pathogenesis of Renal AL Amyloidosis

Shirahama and Cohen (384,400) studied AA amyloidogenesis and demonstrated the importance of mesangial cells and lysosomes in the process of renal amyloidogenesis. Studies in the early 1980s focusing on amyloidogenesis revealed that the mesangium was the first site where amyloid is formed in the glomerulus (407). More recent studies have elucidated the precise mechanisms at play in renal AL amyloidogenesis. When rat or human mesangial cells were incubated with light chains purified from the urine of patients with AL amyloidosis, the light chains were endocytosed into phenotypically changed mesangial cells with a macrophage phenotype (214) and delivered to the mature lysosomal system, where amyloid formation took place (217). The internalization process is clathrin mediated (217). Amyloid formation can be influenced by altering lysosomal function or the mesangial milieu (210,217,408). The process involved in the extrusion of amyloid fibrils into the mesangium by mesangial cells has been studies using scanning electron microscopy. Cellular outpouchings in mesangial cells containing lysosomes are associated with the delivery of fibrils extracellularly (409). Once amyloid is produced and delivered to the extracellular mesangial matrix, activation of metalloproteinases occurs, with eventual destruction of the mesangial matrix and replacement by amyloid (216).

Certain amino acid sequences, as well as posttranslational modifications of the light chains such as glycosylation, dipole moment formation (a change in the degree of polarity of a molecule), and charge-charge interactions, are responsible for the amyloidogenic potential of a given light chain. Some of these alterations include mutations of Arg61 leading to loss of a critical buried salt bridge, mutations of Pro residues in B turns, or replacement of isoleucine (Ile) at position 27b, which enhances

fibrillogenesis by destabilizing the variable portion of the light chains. Replacement of lysine (Lys) 31 is also highly destabilizing and strongly associated with amyloidosis. Replacement mutations in positions 61 (arginine [Arg]-aspartate [Asp]), 31 (Asp-Lys), 36 (tyrosine [Tyr]-phenylalanine [Phe]), and 27 (threonine [Thr]-Ile), among others, may be responsible for the above-mentioned alterations. Other changes in the light chains are also associated with propensity for development of AL amyloidosis (39,66,409–411). Such alterations in the physicochemical composition of a light chain may govern specific interactions with mesangial receptors, potentiating endocytosis into the mesangial cells and eventual amyloid formation (217). The mechanisms involved in AH amyloidosis remain unclear (337–342,396).

AA Amyloidosis (Formerly Secondary Amyloidosis)

Historical Perspective

AA amyloidosis usually arises in the context of an acute-phase response, such as that seen in the inflammatory arthritides, autoinflammatory disorders, chronic infections, and malignancies (278,287,412–423). For this reason, this form of amyloidosis was formerly referred to as "secondary" amyloidosis. However, it is now designated "AA amyloidosis," to reflect its origin from SAA. The amyloid A fibril protein is derived from a larger precursor protein, an acute-phase reactant, termed serum amyloid A or SAA (399,424,425). An association between FMF and the development of AA amyloidosis has been known for decades (418–420,426–430). In recent years, a number of other hereditary autoinflammatory diseases, associated with mutations in genes involved in the inflammatory response of the innate immune system, have been discovered and linked with the development of AA amyloidosis as their major complication (418,423,429–432). Recently, also, a single case of a hereditary form of AA amyloidosis, associated with a mutation in the precursor protein itself, consequent to a mutation in the *SAA4* gene, was reported (433). However, the majority of cases of AA amyloidosis have no apparent familial component. Therefore, AA amyloidosis should be considered in the context of sporadic systemic amyloidosis and, in some cases, as familial amyloidosis associated with hereditary autoinflammatory diseases.

A brief comment should be made in connection with the genetics in familial or hereditary amyloidoses. In patients with hereditary autoinflammatory diseases, the development of AA amyloidosis is associated with genetic mutations in *nonamyloid proteins*. These mutations result in up-regulation of the inflammatory response of the innate immune system, and this inflammation predisposes patients to the development of AA amyloidosis. Later in the section on amyloidoses, we discuss the ever-expanding group of amyloidoses that develop as a consequence of mutations in the *amyloid fibril protein itself* and which are also referred to as hereditary or familial.

Clinical Presentation and Laboratory Findings

AA amyloidosis affects patients of various ages; thus, three series found an age range of 11 to 87 years, with a median of 50 (412–422,427–432). However, in younger patients affected by AA amyloidosis, a hereditary component must be considered (418,427–432) (see below under Hereditary Autoinflammatory Diseases).

There are many conditions that have been associated with AA amyloidosis. Often, the underlying disease has been of long standing and is severe in nature, and this underlying process dominates the clinical picture (412–422,434–437).

The clinical conditions associated with AA amyloidosis include inflammatory arthritides, autoinflammatory diseases, chronic infections, and malignancies. Rheumatoid arthritis is the most frequent, followed by ankylosing spondylitis and other arthritides (434–437). AA amyloidosis has been associated with various autoinflammatory diseases, including periodic fevers, of which FMF is the prototype, as well as granulomatous diseases such as sarcoidosis and inflammatory bowel disorders (Crohn disease) (426–430,438,439). Chronic infections such as tuberculosis, osteomyelitis, bronchiectasis, leprosy, and pyelonephritis; decubitus ulcers; paraplegia; Whipple disease; acne conglobata; cystic fibrosis; intravenous drug use or "skin poppers"; and HIV as well as common variable immunodeficiency, hypogammaglobulinemia, and agammaglobulinemia may be associated with AA amyloidosis (278,287,440–443). Several malignancies, namely, hepatoma, renal cell carcinoma, mesothelioma, Castleman disease, Hodgkin disease, and WM, have also been associated with AA amyloidosis (278,444). Interestingly, between 6% and 13% of patients with AA amyloidosis have no evidence of an underlying inflammatory process (278,445). In the Western world, inflammatory arthritides and inflammatory bowel disorders have gradually replaced infectious diseases as the underlying process most commonly associated with AA (415,417,434–437). In the mid-2000s, in two large Western series (one from the United States and one from the United Kingdom), rheumatoid arthritides accounted for 39% and 64% of AA amyloid cases, respectively, while chronic infections contributed only 13% and 14% of patients (278,446). A large study of patients with rheumatoid arthritis from Japan (447) demonstrated amyloid in 19% of patients. In a series of 68 renal AA amyloidosis patients collected between 1990 and 2005 by French investigators (416), there was an approximately a similar proportion of cases due to chronic infection (40.8%) and chronic inflammation (38%). However, the epidemiology of AA amyloidosis in the Western world has changed further in more recent years. Thus, in both the United States and Northern Europe, over the last 10 years, there has been a decreasing proportion of patients with rheumatologic and infectious causes and an increasing proportion with atypical underlying causes (420,421). During 2000–2009, among patients evaluated at the Boston University Amyloid Center, 59% had "traditional" causes, while 41% had atypical or unknown causes (421). Similarly, comparing the cohort referred to the National Amyloid Centre in London for the period 1992–1996 with the most recent 5-year period, there was a reduction in patients with rheumatoid arthritis from 33% to 19% ($P < 0.038$) and with juvenile idiopathic arthritis from 18% to 2% ($P < 0.001$), but a rise in AA amyloidosis of unknown etiology from 8% to 28% ($P < 0.001$). Overall, however, there has been a remarkable and progressive decrease in patients referred with AA amyloidosis from 32% of all cases in 1987–1995 to 6.8% in 2009–2012 (448). Therapies using disease-modifying antirheumatic drugs (DMARDs) and biologic agents (anti–tumor necrosis factor) for the treatment of rheumatoid arthritis and juvenile idiopathic arthritis have contributed to a sustained decline in the number of new cases (434,449–451). However, outside the Western world,

infectious diseases (in particular tuberculosis) as the underlying cause of AA amyloidosis are more common.

The incidence and prevalence of AA amyloidosis in the general population are largely unknown. For unknown reasons, the incidence varies worldwide and by geographical area, with a higher incidence in Europe than in the United States, even in the setting of the same underlying disease (451). FMF remains an important cause of AA in regions around the Mediterranean (286,419).

Proteinuria and nephrotic syndrome are the most common presenting symptoms. In one recent large series, 97% of patients had significant proteinuria (278). Occasional patients may present with renal failure without significant proteinuria (312,433,452,453). Significant proteinuria has also been associated with a more rapid deterioration in renal function than that seen in patients without significant proteinuria (416,454).

AA amyloidosis usually takes many years to develop. In rheumatoid arthritis, it is at least 2 years, with a mean of 15 years (278,451). Some patients have a preclinical phase of amyloidosis where AA deposits can be detected in tissues without clinical manifestations (436,449). The overall 5-year survival in patients with overt renal disease in AA amyloidosis has been reported to be 40% (278). Median survival after diagnosis was 133 months. However, the prognosis can be markedly improved by control of the underlying inflammatory process (278). Mortality, amyloid burden, and renal prognosis all correlated significantly with the SAA concentration during follow-up, but a relatively favorable outcome was noted in patients with SAA concentrations that remained in the low normal range (<4 mg/L). Interestingly, amyloid deposits regressed in 60% of patients who had a median SAA concentration of <10 mg/L, and survival among these patients was superior to survival among those in whom amyloid deposits did not regress ($P = 0.04$) (278).

Gross and Microscopic Pathology

Renal deposits of amyloid are mostly glomerular, which correlates clinically with proteinuria (278,441). However, in patients without proteinuria, amyloid deposits may be limited to the interstitium and affect predominantly the medulla (278,312,433) or tubules (453) or may be seen around blood vessels (454–456). Less commonly seen patterns of renal amyloidosis include heavy tubular deposits with tubular dysfunction or crescentic glomerulonephritis (457). In one large series of 68 patients (416), the distribution pattern of glomerular amyloid deposits was mesangial segmental (14.7%), mesangial nodular (26.5%), mesangiocapillary (32.3%), and hilar (26.5%). In the same study, glomerular amyloid was observed in 80.9% of cases and vascular amyloid without glomerular involvement in 19.1%. A similar amyloid distribution, glomerular versus vascular, was seen by Uda et al. (454). Tubular atrophy, abundance, and the distribution pattern of glomerular amyloid deposits at the time of biopsy were independent predictors of renal outcome. In one study of patients with glomerular involvement, renal function deteriorated rapidly, regardless of the disease state, and most patients received hemodialysis (454). In contrast, in patients with purely vascular involvement, renal function did not deteriorate significantly (416,454).

Autopsy studies have shown that amyloid can be present in nearly every organ (456). The kidneys are almost always involved (278,412,447–460). Gastrointestinal tract involvement is seen in approximately 20% of patients, while, in contrast, the testes are much more frequently involved (87%) (417,460). However, in one recent biopsy study from Japan, gastrointestinal amyloid showed a better correlation with renal involvement than abdominal fat (454,460). Amyloid goiter and spleen, liver, adrenal gland, and pulmonary involvement are relatively less common (417,421,461). Myocardial involvement has generally been rare (278,421).

It is generally agreed that AA amyloidosis can be reliably diagnosed by immunohistochemical methods, in virtually all cases, in both frozen and paraffin sections (278,440,462) (see Fig. 22.21A). There are also several case reports of codeposition of AA and AL. Petterson et al. (317) reported a patient with ankylosing spondylitis with renal glomerular AL and vascular AA amyloidosis. Similar consideration must also be given to patients with WM, who typically develop AL. However, in some patients, WM may be associated with AA amyloidosis (440). Similar, seemingly paradoxic, development of an AL amyloid type was reported in ankylosing spondylitis and in Crohn disease, both of which are a common clinical setting for AA (313,354,463).

Hereditary Autoinflammatory Diseases

Historical Perspective

As a group, the hereditary autoinflammatory diseases are associated with apparently unprovoked attacks of recurrent inflammation (286,418,422,426–430). In contrast to autoimmune diseases, these attacks are not associated with significant levels of autoantibodies or antigen-specific T cells. While derangement in the adaptive immune system is involved in autoimmune diseases (e.g., systemic lupus erythematosus, rheumatoid arthritis), genetic variants in the *innate* immune system, including Mendelian and genetically complex disorders, are associated with the autoinflammatory syndromes (418,464,465). Accordingly, the inflammatory attacks are mediated by neutrophils and macrophages, which are part of innate immunity. Although the exact triggers of these inflammatory attacks are unknown, they often appear to be associated with stress, immunization, or trauma. Therefore, it has been postulated that gene-environment interactions may play an important role in the pathogenesis of the hereditary autoinflammatory diseases. Advancements during recent decades have led to the development of a new classification of these diseases into monogenic and polygenic/complex autoinflammatory diseases (418,464,465). The former encompass hereditary periodic fever syndromes. Examples of polygenic and complex autoinflammatory disorders include Behçet disease, Crohn disease, sarcoidosis, and sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease) (recently reviewed by Ombrello and Aksentijevich (418)). Behçet disease has been associated with AA amyloidosis in up to 5% of patients (466). It is estimated that AA amyloidosis develops in approximately 1% of patients with Crohn disease in the United States and up to 3% of patients in Northern Europe, and complications of amyloidosis are a major cause of mortality (418). In contrast, AA amyloidosis in ulcerative colitis is extremely rare. Unfortunately, the pathogenesis of inflammatory bowel disease is poorly understood, but many studies have suggested an abnormal mucosal immune system, both innate and adaptive, as a contributing factor.

Periodic fever syndromes, which are monogenic autoinflammatory diseases, are characterized by recurrent fevers and systemic inflammation. Although the role of genetic factors in the development of AA amyloidosis in FMF was suspected, the underlying mechanisms have been discovered only relatively recently (427,429,467–469). At the same time, several other chronic inflammatory diseases, which may be complicated by the development of AA amyloidosis, have also been shown to have genetic components (469–471). Most patients with hereditary periodic fevers, including FMF, have mutations in the pyrin, cryopyrin, or tumor necrosis factor (TNF) receptor genes (469–471). Their products modulate the activity of apoptotic proteins and signal transduction pathways, playing a crucial role in the inflammatory response of the innate immune system. Thus, collectively, these diseases can be considered to be a consequence of an inborn error of inflammation. Apart from FMF, there are several other periodic fevers that have recently been clinically, as well as genetically, characterized: tumor necrosis factor receptor 1–associated periodic syndrome (TRAPS), cryopyrin-associated periodic syndrome (CAPS), and others (469–471). As a group, these disorders are referred to as "Infevers," and recently, a database (http://fmf.igh.cnrs.fr.infevers/) was established for them (469). FMF is autosomal recessive; all others are autosomal dominant and rare. A thorough diagnosis is warranted, because clinical and therapeutic management is specific for each of these diseases (472–475).

Several of the hereditary autoinflammatory diseases carry an increased risk for the development of AA amyloidosis. Familial AA amyloidosis develops in the context of mutations in genes for nonamyloid fibril proteins that play a permissive role in the development of amyloid (467–471). In contrast, other familial amyloidoses are associated with a genetic defect leading to amino acid substitutions in the amyloid fibril precursor proteins themselves (please see below under Hereditary Amyloidoses).

Familial Mediterranean Fever

FMF is by far the most common form of the nephropathic familial amyloidosis. It occurs in ethnic groups from around the Mediterranean basin and in their descendants: non-Ashkenazi (Sephardic) Jews of North African or Middle Eastern descent, Armenians, Middle Eastern Arabs, and Turks (418,422,429,469,472–474). However, as genetic testing becomes more frequent, clinical cases in individuals of non-Mediterranean ancestry are increasingly reported (418,469). It is speculated that heterozygotes carrying mutant alleles have a selective survival advantage owing to their heightened inflammatory state, which helps them clear a putative endemic Mediterranean pathogen(s) (475). The gene responsible for this febrile disorder, *MEFV* (mapped to the short arm of chromosome 16), encodes pyrin (418,467,468).

Clinical Presentation and Laboratory Findings

FMF is characterized by regular, unpredictable, and painful febrile episodes lasting 1 to 4 days accompanied by sterile peritonitis, pleuritis, synovitis, or an erysipelas-like erythema involving the lower extremities. The symptoms related to synovitis may be prolonged. No specific inciting factor has been identified (418,422,429,475). The symptoms resolve without treatment and with no apparent ill effects, until amyloidosis supervenes. AA amyloidosis is the main and potentially lethal complication of the disease. It affects most patients before 40 years of age, but has been reported in children as young as 5 years old (418,422,429).

Renal involvement is the principal clinical manifestation of AA amyloidosis in patients with FMF (418,422,429). Proteinuria is typically followed by nephrotic syndrome and uremia. Proteinuria during the nephrotic stage may be massive, and the course may be complicated by renal vein thrombosis with abrupt deterioration of renal function. The uremic stage supervenes as glomerular amyloid occludes the tufts, and hypertension is found in up to 50% of patients at this stage. The duration of clinical renal disease, from the onset of proteinuria to terminal renal failure, varies from 2 to 13 years (476,477). Development of amyloid nephropathy may follow a period of active disease (phenotype I) or, on rare occasions, may be the presenting manifestation (phenotype II) (427,429).

Amyloidosis appears to be the inevitable consequence of untreated disease, but the prevalence of AA in inadequately treated FMF patients varies with population group. In untreated patients, amyloidosis occurred in 60% of Turkish patients and in 27% of non-Ashkenazi Jews (476). Amyloidosis was the cause of death in virtually all autopsied patients with FMF who were not treated with colchicine (476).

Gross and Microscopic Pathology

Amyloid deposits in FMF are distributed throughout the body in small vessels. In autopsy kidneys, the glomeruli are always extensively involved with gross replacement of the tufts. Amyloid deposits are also seen in dense rings around the tubules. Interstitial deposits are inconstant, but when present, they appear to preferentially accumulate in the medulla (476,478,479). Glomerular amyloid deposits begin in the mesangium and spread via the subendothelial space to involve the tuft, in the same fashion as other forms of systemic amyloidosis. Although, in a patient with FMF, proteinuria is presumptive evidence of amyloidosis, other renal pathologies should be considered, in particular in patients treated with colchicine. Said et al. (478) performed biopsies on 15 patients with FMF, principally to elucidate the cause of proteinuria, and only seven patients had amyloidosis, while others had different pathologies. Similar findings were reported by Kukuy et al. (480). In their series, among 27 patients with FMF and proteinuria, almost 50% of patients had nephropathy other than amyloidosis, including entities with a more favorable outcome. Therefore, the authors highly recommended performance of a kidney biopsy in patients with FMF and proteinuria of more than 0.5 g/24 h. Interestingly, increased incidence of vasculitides and Henoch-Schönlein disease has been reported in FMF patients (481). The sensitivity of renal biopsy for the detection of amyloidosis in FMF was 88% in one large series, followed by rectal biopsy at 75%, liver biopsy at 48%, and gingival biopsy at 19% (418). The reported sensitivity of testicular biopsy is about 87% (482).

AA amyloidosis frequently involves the endocrine system, and endocrine dysfunction is increasingly recognized (479,480) including that of the thyroid gland, testicular amyloid deposits, and adrenal dysfunction. Testicular involvement is frequently seen in systemic AA (and AApolipo AI amyloidosis—discussed below), and since it may affect young adults, its

clinical relevance is increasing (482–484). Testicular involvement by amyloidosis may be associated with testicular enlargement and lead to abnormal spermatogenesis and secondary infertility (482–484). In patients with known amyloidosis or identifiable risk factors (e.g., FMF), sperm cryopreservation and early sperm retrieval may be considered.

Etiology and Pathogenesis of AA Amyloidosis

The amyloid fibril protein in sporadic AA amyloidosis, FMF, and other autoinflammatory diseases is derived from a truncated apolipoprotein, SAA (418,423–425). SAA is highly conserved among species and is a major acute-phase reactant. Its normal physiologic function is unknown, but it has been speculated that SAA plays a role in inflammation, pathogen defense, high-density lipoprotein (HDL) metabolism, and cholesterol transport (418,425). AA amyloidosis develops in association with an enhanced and prolonged inflammation that leads to up-regulated production of its precursor and, subsequently, to its incomplete degradation, misfolding, and deposition in the tissues. Other apolipoproteins (apolipoprotein AI, AII, and AIV) have also been shown to be associated with amyloidosis, both hereditary (apolipoprotein AI and AII) and sporadic (apolipoprotein AIV) (discussed later in the section). In case of apolipoproteins AI and AII, amyloid is derived from various mutants; thus far, no mutation has been observed in apolipoprotein AIV.

The human *SAA* genes are located on chromosome 11(418,425). In humans, SAA is expressed by three different genes: *SAA1*, *SAA2*, and *SAA4*. *SAA3* is a pseudogene, and *SAA4* is constitutively expressed, while SAA1 and SAA2 are acute-phase reactants and are involved in AA amyloidogenesis. These isoforms are synthesized by the liver in response to proinflammatory cytokines (IL-1, IL-6, TNF-α), and their levels can rise >1000-fold during inflammation. In patients with FMF, serum levels of SAA are elevated two to three times normal under basal conditions and may rise >50 times during febrile episodes (418,467,469).

Under physiologic conditions, SAA is secreted as a 104 amino acid protein, which is completely degraded. In contrast, in AA amyloidosis, there is incomplete degradation of SAA, with the C-terminal portion being cleaved, and an accumulation of the intermediate products. This results in SAA fragments containing only 66 to 76 amino acids, which subsequently polymerize into amyloid fibrils. During fibrillogenesis, the amyloid protein fragments bind proteoglycans and amyloid P component. Thus, AA amyloidosis is a result of the interplay of three major mechanisms (418,425,485–487): increased production of SAA, its proteolysis, and participation of the extracellular matrix. It is postulated that proteolytic processing of SAA to AA protein takes place within macrophages (418,425,485–487). Experimental studies have shown that, in the kidney, mesangial cells are involved in the processing of AA (as well as AL) (384,400,409). It is, as yet, unknown why some patients with inflammatory disease and high levels of SAA develop amyloidosis, while others do not (418). It is postulated that other factors must also be implicated, such as genetic *SAA* polymorphisms (see below) (418) and yet-unknown factors that affect the degradation of serum amyloid A to AA protein (485–487). To this end, there is evidence that certain *SAA* polymorphisms may be associated with an increased risk for the development of AA. Thus, among Caucasians, the incidence of AA appears to be increased in persons who have the SAA1α.α genotype; however, in Japan, the SAA1.γ allele carries an increased risk. Moreover, specific genetic mutations seen in several autoinflammatory diseases also carry an increased risk for the development of amyloidosis. The *FMF* gene encodes pyrin, a member of the pyrin gene family, which encompasses several related genes involved in autoinflammatory diseases by affecting apoptotic and inflammatory signaling pathways. Interestingly, in 2009, Murphy et al. reported the first case of renal AA amyloid derived from a mutated SAA4 protein, which is constitutively expressed (433). This patient had no antecedent history of chronic inflammatory or neoplastic processes. The SAA4 nature of the amyloid was first discovered by tandem mass spectrometry and subsequently confirmed immunohistochemically. Interestingly, only wild-type SAA4 was detected by mass spectrometry in the patient's serum, and its concentration was within normal limits. The authors postulated that the mutation (W22G substitution) had a profound effect on the stability of SAA4, rendering it amyloidogenic. The role of a genetic component, rather than overproduction and incomplete digestion, also emerged recently in a single case of hereditary amyloidosis derived from β_2-microglobulin (please see later in this segment). Amyloid formation is a complex process, of which only certain features have been discovered and/or elucidated.

Data derived from an animal model of AA amyloidosis show that amyloidogenesis is preceded by changes in the composition of the extracellular matrix. There is increased deposition of GAGs and amyloid P component accompanying amyloid fibril protein deposition (485–490). Interestingly, the extracellular matrix, in particular glycosaminoglycans and the amyloid P component, appears to be involved in fibril formation in all types of amyloidosis thus far studied (489,490). The role of these ancillary components is unclear. Several functions have been proposed, including the role of facilitating aggregation and protein misfolding, leading to fibril formation, substrate adhesion, and protection from degradation (485–487,489). Protein aggregation has recently developed into an area of intensive research, since inhibition of aggregation has been targeted as a potentially useful approach to decrease the amyloid burden during disease (475). Amyloid P component, a glycoprotein related to C-reactive protein, can be detected in amyloid deposits by scintigraphy. This feature has been successfully used by some amyloid centers in Europe to assess the kinetics of amyloid deposition following treatment (278).

Nonimmunoglobulin and Non-AA Amyloidoses

In this segment, apparently sporadic as well as hereditary amyloidoses are considered. The former include amyloidosis derived from leukocyte chemotactic factor 2 and apolipoprotein AIV; amyloidosis derived from β_2-microglobulin has also been largely acquired, with the exception of a single recently reported hereditary case. Among the hereditary systemic amyloidoses, discussed here are amyloidoses derived from transthyretin, fibrinogen, apolipoproteins AI and AII, lysozyme, gelsolin, and cystatin.

Amyloidosis Derived from Leukocyte Chemotactic Factor 2, ALect2

Since the publication of the last edition of this book, a new form of systemic amyloidosis has been identified, which is derived from leukocyte chemotactic factor 2, designated ALect2 (491). A significant number of renal biopsies with previously unknown types of amyloid were subsequently shown

to be ALect2 (284,347,491–493). It is currently believed that ALect2 represents the third most common form of renal amyloidosis (347,493). For example, in a recently reported large series of 445 recent cases of renal amyloidosis, 13 biopsies (2.9%) were shown to contain ALect2 amyloid (284). For comparison, in the same series, AA was identified in 33 (7.4%) cases, and amyloid derived from fibrinogen (AFib) was diagnosed in 5 (1.2%) cases and apolipoprotein in 3 (0.7%) cases. Thus, AA amyloid, the second most common type of renal amyloidosis, was only three times more frequent than ALect2.

Clinical Presentation and Laboratory Findings

ALect2 amyloidosis has been characterized by renal involvement, with extensive deposits of amyloid in the glomeruli, interstitium, and extraglomerular vessels (347,491–493). Clinically, there is renal function impairment and varying amounts of proteinuria. However, Holanda et al. (494) reported a case of renal ALect2 without proteinuria at presentation or on subsequent follow-up. Interestingly, in a recently reported series of hepatic amyloidosis cases (495) from a major referral center, ALect2 represented the second most common type of amyloidosis, involving liver in 23% of their cases (21/92). While this large proportion of hepatic ALect2 amyloid may be due to referral bias, the authors propose that ALect2 involves the liver at least as frequently as the kidney. Fix et al. (496) reported a first case of ALect2, with primary liver involvement, resulting in acute liver failure. The patient developed hepatic encephalopathy with confusion and rapidly worsening renal function, as a result of which a combined liver and kidney transplantation was performed; this was the first such procedure involving ALect2. In a series of 30 patients with ALect2, no patient had clinical or laboratory evidence of involvement of the heart or nerves (493). However, more recently, a single patient with presumed ALect2 heart involvement has been reported (496).

Thus far, there have been no reports of a family history or condition that is known to underlie other amyloidoses. No increased levels of plasma Lect2 have been documented (492).

Gross and Microscopic Pathology

In general, ALect2 should be suspected if there is extensive interstitial involvement. Thus far, reported renal biopsies have shown fairly advanced involvement of the glomeruli, tubular basement membrane, interstitium, and extraglomerular vessels. Interestingly, in the recently reported series of 30 patients (347,492,493), deposition of ALect2 was predominantly interstitial and vascular, with relative sparing of the glomeruli. Holanda et al. also reported a kidney biopsy that showed amyloid in the tubular basement membrane, vascular walls, and interstitium along with a relatively mild segmental glomerular involvement (494).

ALect2 is truly systemic and, in addition to kidney, hepatic, splenic, colon, and adrenal involvement, was also discovered incidentally (493). In the liver, unusual globular parenchymal deposits of amyloid are typically seen.

Initial reports suggested that ALect2 reacts well with the corresponding antibody (491), but antigen retrieval may be needed for paraffin sections. However, recently, it was suggested that immunohistochemistry positive for ALect2 may not be sufficiently specific to determine the amyloid type. This is based on the detection of a minor component of ALect2 reactivity by immunohistochemistry in kidney biopsies with AL as the main component (363). Whether ALect2 plays a pathogenic role in amyloidogenesis in these AL patients will require further investigation. Caution in interpretation of the immunohistochemistry results is also advised.

Etiology and Pathogenesis

Although ALect2 amyloid has been diagnosed predominantly in patients of Hispanic origin and in some patients from the Punjab, thus far, there is no evidence that ALect2 amyloidosis is hereditary (492,493). However, the available molecular studies are relatively limited (491,492,497). Amino acid sequence data from the amyloid protein and the cDNA data thus far reported showed no mutation. Thus far, also, ALect2 amyloidosis has been diagnosed in adult patients only, and no instance of a family history has been reported.

The amyloid protein appears to be composed of the entire 133-amino-acid LECT2 protein (491,498). In the secreted protein, there is a polymorphic G allele that encodes valine at position 40. Interestingly, each patient studied, thus far, was homozygous for the G allele (492). It is, therefore, feasible that genetic variation in the *LECT2* gene may play a role. Computer modeling suggests that Lect2 has a β-domain, similar to other amyloidogenic proteins. Theoretically, replacement of isoleucine (A allele) with valine (G allele) could destabilize the protein and account for the amyloidogenic propensity of this Lect2 variant. Human Lect2 (aka chondromodulin-II) is a normal serum protein, encoded by the *LECT2* gene, which was mapped to chromosome 5q31.1-q32 by fluorescence in situ hybridization. This region contains a cluster of cytokine genes including IL-4, IL-5, and IL-9. The secreted Lect2, a 16-kDa protein, is a chemokine and a growth factor, known for being a chemotactic factor for neutrophils and for stimulating the growth of chondrocytes and osteoblasts; it may also be involved in the regulation of repair after damage. Lect2 appears to be synthesized predominately in the liver, but it is also expressed in other tissues, including the testis, vascular and endothelial smooth muscle cells, and the kidney. Lect2 is a downstream target of β-catenin, which plays an important role in various aspects of liver biology, including the pathogenesis of liver cancer. Lect2 appears to be underexpressed in hepatocellular carcinoma (498,499) and may become a candidate prognostic marker for this tumor; therapeutic strategies targeting Lect2 expression as a therapy for human hepatocellular carcinoma are also being investigated. However, the pathogenesis of Lect2 amyloidosis in the liver, as well as in other organs, is unclear. It has been suggested (492) that ALect2 may be a consequence of an inflammatory process that leads to increased synthesis of the potentially amyloidogenic valine-40 variant in individuals homozygous for the G allele. Interestingly, a polymorphism in this gene may be associated with rheumatoid arthritis.

Amyloidosis Derived from Apolipoprotein AIV, AApoAIV

Apolipoprotein AIV–derived amyloid (AApoAIV) was first reported in 2001 by Bergstrom et al. (500). A cardiac specimen from an elderly patient was found to contain two different types of amyloid: one derived from wild-type transthyretin and another from wild-type apolipoprotein AIV. In a subsequent publication (349), the authors established the systemic distribution of amyloid deposits and the complete amino acid sequence of the 70-residue N-terminal fragment of apolipoprotein AIV forming amyloid fibrils. There were no mutations in the nucleotides of the *APOA4* gene cloned from the patient's

genomic DNA. Over a decade later, the issue of AApoAIV amyloidosis was revisited with the emergence of additional cardiac and renal cases. In 2012, Sethi et al. (313) reported renal AApoAIV amyloidosis in a 52-year-old male with increased urinary frequency, a gradual loss of renal function, but no significant proteinuria. Four additional cases of AApoAIV with amyloid deposits in renal and cardiac tissues, from the same institution, were reported in the same year (501). Three other cases, in 82-, 62-, and 57-year-old males, were reported from another institution in the United States (502), and three additional cases were apparently also diagnosed in the United Kingdom (503). Renal biopsies showed large amounts of amyloid restricted to the medulla/interstitial compartment, with no involvement of glomeruli or vessels. Thus far, there is no evidence of family history or mutation in the *APOA4* gene. In cases reported by Sethi et al. (313) and Prokaeva et al. (502), DNA analyses detected sequence variants representing common polymorphisms of the *APOA4* gene. No information was reported regarding other cases.

ApoAIV is a 46-kDa glycoprotein that is primarily synthesized by the enterocytes of the small intestine and is important in the absorption, transport, and metabolism of lipids. The plasma concentration varies, depending on nutritional status, and increases with age. Apolipoproteins have been known to be associated with amyloidoses. Thus, ApoAI and ApoAII have been associated with several familial systemic amyloidoses, while serum amyloid A is the precursor protein of fibrils in secondary or reactive (AA) amyloidosis. While the presence of mutations in the familial forms may confer amyloidogenicity, detection of a wild-type amyloid suggests that apolipoproteins may share structural features that render them amyloidogenic.

Hereditary Amyloidoses Resulting From Amyloid Fibril Protein Precursor Mutations

Historical Perspective

Hereditary systemic amyloidosis is a diverse group of diseases that occur less frequently than AL or AA. In these amyloidoses, the structure of the amyloid precursor protein is altered by a mutation, and this factor is considered pivotal in amyloidogenesis (267,303,310,504). The first mutation identified in hereditary amyloidosis resulted from coding sequence missense mutations in the *TTR* (transthyretin) gene (504). Subsequently, several additional genes (and many more variants) have been shown to be involved in hereditary amyloidoses. Thus, hereditary amyloidoses show both genetic heterogeneity (multiple genes) and allelic heterogeneity (multiple mutations in the given gene are able to cause the disease). Although they are all inherited as autosomal dominant traits, due to variations in the degree of genetic penetrance, a family history may not be present. Moreover, the phenotype can be similar to AL or AA (365). Therefore, diagnosis can be challenging. While certain hereditary amyloidoses are concentrated in particular geographic locations, or affect defined ethnic groups, many patients throughout the world have been diagnosed with these various diseases (504). Thus, hereditary amyloidosis should not be excluded in a given patient based on ethnicity alone. Currently, in keeping with the modern classification of amyloidoses, these disorders are named after the amyloid fibril protein. Among these diseases are amyloidoses derived from transthyretin (ATTR), fibrinogen-α chain (AFib), apolipoprotein AI (AApoAI), apolipoprotein AII (AApoAII), gelsolin (AGel), lysozyme (ALys), and cystatin (ACys) (267,504).

Recently, single reports were published of mutations in the *SAA4* gene and the B*2M* gene (β_2-microglobulin) (discussed earlier and below in this section, respectively) (369,433). Moreover, among two recently reported systemic amyloidoses, AApoAIV and ALect2, the presence of a mutation has not so far been detected, but it has not been ruled out either (491,492). While several of the currently known familial disorders are distinctly neuropathic or cardiopathic, virtually all of them can affect the kidneys, although, in some of these amyloidoses, renal deposits may be clinically silent. Nephropathy is a common feature of amyloidosis caused by mutations in fibrinogen A α (alpha)-chain, lysozyme, apolipoprotein AI (ApoAI), and apolipoprotein AII (ApoAII) (504). Cardiomyopathy is commonly seen in transthyretin amyloidosis and in some patients with ApoAI amyloidosis. Neuropathy may be a feature of transthyretin and ApoAI amyloidosis (366,504).

Although generally considered to be rare, in aggregate, hereditary amyloidosis is relatively common. At Boston University, 10% of patients were diagnosed with familial amyloidosis, with 85% of the familial amyloidosis due to ATTR and 5% due to AFib (288). In contrast, in the United Kingdom, AFib is the most frequent hereditary amyloidosis (365). The low incidence of these disorders may be a consequence of underdiagnosis. Although individually rare, it is imperative that these amyloidoses are properly diagnosed because of the implications for patient management, including appropriate counseling, prognostication, and treatment (please see under treatment).

The dangers of misdiagnosing of hereditary amyloidoses as AL have been discussed in the literature. In one such study, an amyloidogenic mutation was present in 34 out of 350 patients with systemic amyloidosis (9.7%): most often, mutations were detected in the genes encoding fibrinogen A alpha chain (18 patients) and transthyretin (13 patients) (365). In all 34 of these patients, the diagnosis of hereditary amyloidosis was confirmed by additional investigations. A low-grade monoclonal gammopathy was detected in 8 of the 34 patients (24%). Comenzo et al. (505) reported similar findings where 6% of screened patients had both a monoclonal gammopathy and a hereditary variant. Similar findings were also reported by others (463). These results justify the need for screening for hereditary variants and highlight the importance of a correct diagnosis of the amyloid type based on the examination of the amyloid protein in deposits.

Additional Comment on the Nomenclature of Hereditary Amyloidoses

The amyloid nomenclature is discussed earlier in this chapter. Here, additional comments pertaining to hereditary amyloidoses are included.

Until the amyloid fibril proteins and their precursors were identified, familial amyloidoses were classified by clinical and pathologic phenotype (267,271,504). However, in the modern nomenclature, the amyloidosis type is named after the protein; for example, AFib or ATTR amyloidosis, and the use of the older clinical terms (e.g., familial amyloid polyneuropathy) is discouraged. Variants are named according to the mutation in the gene for the protein, for example, ATTRV30M or ALysI56T. The first amino acid of the mature, processed

protein is numbered as amino acid number one (267). While this numbering system reflects the amino acid sequence in the *secreted* proteins, such as amyloid proteins, it does not take into consideration the signal peptides and propeptides that are cleaved from the amino terminus after translation, as the protein is being processed by the cell for secretion. Although this current amyloid nomenclature has been widely used in the amyloid field, such a designation of variants is inconsistent with the recommendations of the Human Genome Variation Society (HGVS). The latter has proposed a standard nomenclature for variation, both at the nucleotide and at the protein level (www.hgvs.org/mutnomen/). While a detailed description is beyond the scope of this chapter, the reader is referred to the HGVS Web site (http://www.hgvs.org/) and other references for additional reading (497).

Another issue awaiting address is that of the distinction between hereditary amyloidoses caused by a mutation in the amyloid fibril itself and familial AA amyloidoses that occur in a "familial" setting owing to various mutations in *nonamyloid* proteins.

Amyloidosis Derived From Transthyretin, ATTR

Historical Perspective

ATTR is the most common form of systemic hereditary amyloidosis (504). Transthyretin, a prominent plasma protein formerly known also as prealbumin, is a carrier protein for thyroid hormone and retinol binding protein/vitamin A (504). Transthyretin is synthesized primarily by hepatocytes, but small amounts are also synthesized by the choroid plexus and the retinal pigment epithelium of the eye. Transthyretin is composed of 127 amino acids and 4 monomers form a tetramer that circulates in the blood as a 56-kD transport protein. Wild-type transthyretin has extensive β-pleated sheet structure and is, therefore, a prime candidate for amyloid formation; indeed, wild-type TTR can undergo fibrillogenesis in older patients, who develop senile systemic amyloidosis. Moreover, post–liver transplantation (please see below), there is also a continuing deposition of amyloid derived from the wild type.

The human *TTR* gene, located on chromosome 18, has been shown to be affected by >100 different mutations, most of which (but not all) are pathogenic. The most frequent, worldwide, is the mutation leading to the Val30Met substitution (Met30). There are well-established foci of familial ATTR in Portugal, Sweden, and Japan. However, the disease has been documented in many countries. Almost 4% of African Americans carry a mutation that probably originated on the west coast of Africa and leads to the substitution of isoleucine for valine at position 122 (V122I) (506). Many of the other mutations have been described in single families or single individuals, and due to incomplete penetrance of the genetic defect, many cases appear "sporadic." However, at least in some cases, a more detailed investigation may demonstrate the genetic basis of the disease. ATTR was originally named "familial amyloidotic polyneuropathy" (FAP) because the first mutation discovered, Val30Met, is associated principally with neuropathy.

Clinical Presentation and Laboratory Findings

The age at onset and the phenotype may vary between different kinships. In general, males are more often affected than females. There are also geographic differences. The same variant (Met30) produces an earlier age of onset in Portuguese, and a later age of onset in Swedish, endemic foci (316,504). A low penetrance in carriers of the mutation has been observed, and therefore, the family history may be absent (365,504); occasional reports of apparently sporadic ATTR cases have also been noted (504). In elderly patients, wild-type transthyretin may form amyloid, which shows cardiac tropism. Senile systemic amyloidosis affects an estimated 20% to 25% of people over the age of 80 (383,504,506,507).

Despite being inherited, the disease is generally not clinically apparent until middle or later life.

Although polyneuropathy and cardiomyopathy are the major clinical manifestations, nephropathic and ocular forms have also been reported (316). Microalbuminuria may be the first stage of clinical ATTR nephropathy and is premonitory of neuropathy (316). The most frequent form of presentation of ATTR nephropathy is nephrotic proteinuria with renal dysfunction (316). Some mutations appear to be associated with renal impairment without proteinuria (504).

Testing for familial amyloidosis should be considered in any inconclusive cases, and DNA analysis is mandatory for supporting the diagnosis. Serum screening for variants of transthyretin by isoelectric focusing and mass spectrometric characterization of variants are available. However, this serum-based test is limited to TTR amyloidosis and does not cover amyloidosis due to the other genes. In the past, multiple restriction fragment length polymorphisms (RFLPs) have been used to diagnose ATTR. At present, ATTR diagnosis is based on direct DNA sequencing, typically using DNA obtained from peripheral blood cells. Transthyretin sequence analysis can be obtained from a number of commercial laboratories and amyloid treatment centers (504).

Gross and Microscopic Pathology

Amyloid deposits are systemic but typically affect peripheral nerves and myocardium, depending on the phenotype. Varying degrees of renal involvement have been reported, with primarily glomerular deposits, but in some patients, amyloid may be limited to the interstitium in the medulla (316). End-stage renal disease may develop (315,316). There are amyloid deposits in the systemic vasculature. Preamyloidotic deposits can be detected in nerves of some carriers without clinical evidence of the disease (298). Mixed deposits of amyloid, that is, derived from two different proteins (transthyretin and apolipoprotein AIV), have also been reported in the heart (500).

In ATTRV122I patients, the heart is the most severely affected organ, but amyloid is also variably seen in the blood vessels of multiple tissues, including the kidney, adrenal gland, urinary bladder, prostate, lung, various segments of the gastrointestinal tract (including the tongue), thyroid, spleen, and adipose tissue. Interstitial amyloid can also be seen in the kidney and in the adrenal gland, bladder, and prostate as well as in the gastrointestinal tract including the liver and spleen (508). Senile systemic amyloidosis primarily affects the myocardium (frequently referred to as "senile cardiac amyloidosis"), but there is also systemic involvement of the vessels and, frequently, symptomatic involvement of the lungs and carpal tunnel; other sites, frequently seen at autopsy, are usually clinically silent ((508), Picken—unpublished observation). In the kidney, small interstitial, predominantly medullary amyloid deposits may be seen (Picken—unpublished observation).

Etiology and Pathogenesis

It is believed that the presence of a mutant form of the amyloid protein precursor is pivotal in the development of amyloidosis. However, there is considerable variation in affected gene penetrance and the mutant phenotype (316,509). Similar variations in phenotype have been observed in other hereditary amyloidoses (see below, (509)). Several modifiers (genetic and/or environmental factors) of precursor protein gene mutation effects have been hypothesized.

It is also hypothesized that, while accumulation of the amyloid fibrils in the extracellular space is ultimately detrimental to tissue and organ function, it is the process of amyloid fibril formation itself that is toxic. It is postulated that it is the nonfibril intermediates and oligomeric aggregates formed during fibrillogenesis, rather than the mature fibrils themselves, that are proteo- and cytotoxic (Fig. 22.24) (510). Functional TTR forms a tetramer that is involved in the transport of thyroxine and the vitamin A (retinol)-binding protein complex. In vitro studies have demonstrated that the presence of TTR mutants compromises the stability of the tetramer and, thereby, leads to the formation of monomers, which are prone to misfolding and aggregation. Targeted therapies, currently under testing, have focused on small molecules that kinetically stabilize the tetramer, thereby inhibiting TTR amyloid fibril formation. While, in hereditary ATTR, gene mutations and the ensuing amino acid substitutions clearly destabilize the transthyretin tetramer, factors involved in wild-type ATTR accumulation are unknown; however, there is a striking association with advanced age. (Liver transplantation—please see under treatment.)

Amyloidosis Derived From Fibrinogen A α-chain (AFib)

Historical Perspective

Amyloid derived from a mutant fibrinogen A α-chain (AFib) was discovered by Benson et al. in 1993 (310).

It is characterized by a predominantly renal involvement and appears to be the most common type of hereditary renal amyloidosis in Europe and, possibly, also in the United States (504,511–513). Kindreds have been identified in the United Kingdom, Mexico, the United States, France, and Asia (280,303,305,504,511–513). A family history of renal disease is frequently absent, and the disease is characterized by variable penetrance. However, in one series, a high penetrance was reported (513). De novo mutation has been documented (280), in a child.

Clinical Presentation and Laboratory Findings

Age at presentation ranges from the third to the eighth decade (median 58 years) (511). In one large series, all patients presented with renal symptoms of proteinuria, hypertension, and mild renal failure. Kidney failure, from initial presentation to end-stage renal failure and dialysis dependence, may be fairly rapid, within 1 to 5 years. In a large series, median time from presentation to end-stage renal diseases was 4.6 years, and the estimated median patient survival from presentation was 15.2 years

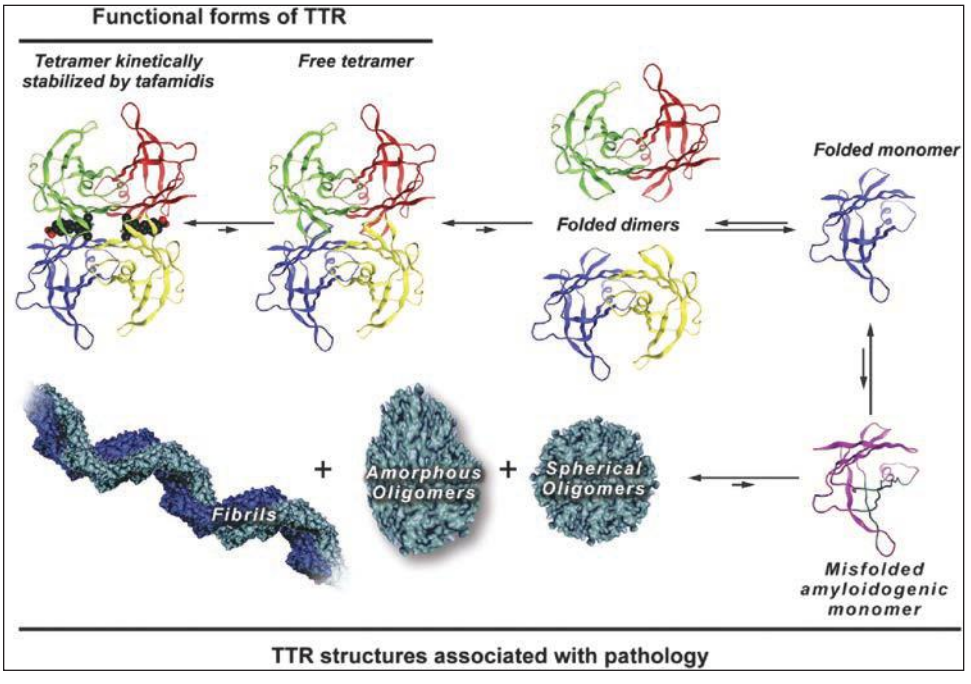

FIGURE 22.24 Proposed mechanism involved in TTR fibrillogenesis. A functional form of TTR circulates as a tetramer; however, in the presence of a mutant protein, the stability of the tetramer is compromised, and it dissociates into monomers. Partial unfolding of the monomers produces an amyloidogenic intermediate that is aggregation prone and can misassemble to form a variety of aggregates, such as spherical oligomers, amorphous aggregates, and fibrils. Currently tested treatments involve compounds that can stabilize the TTR tetramer by slowing its dissociation into monomers and, thereby, abolish aggregation. (From Bulawa CE, Connelly S, Devit M, et al. Tafamidis, a potent and selective transthyretin kinetic stabilizer that inhibits the amyloid cascade. *Proc Natl Acad Sci U S A* 2012;109:9629.)

(511–513). Initial reports suggested that AFib is associated with a relatively slow progression of amyloid deposition, when compared with AL, and that clinically significant extrarenal disease is rare. Although a high prevalence of atherosclerotic cardiovascular disease was noted, it was believed to be secondary to chronic kidney disease. However, more recent series published by Stangou and Tavares reported a high incidence of previously unreported visceral, vascular, cardiac, and neurologic involvement in AFib (512,513). Thus, AFib appears not to be solely nephropathic but, rather, a more systemic disease with a diverse and complex phenotype (512,513). In particular, cardiovascular involvement may be underdiagnosed, and more recent reports have suggested that the cardiovascular atheromatous disease appears to predate proteinuria or renal failure by many years; also, the reports indicate that there is a strong family history of coronary/vascular disease in these patients (512,514). While DNA analysis by RFLP is available for some of the mutations, in many patients direct nucleotide sequencing may be needed to conclusively diagnose the genetic defect (504).

Gross and Microscopic Pathology

AFib shows a remarkable tropism for the kidney. The histology is very characteristic, showing glomerular enlargement with near-total replacement of the glomeruli by amyloid, with little or no vascular or interstitial amyloid (511–513). However, recently, Tavares (513) also detected cortical interstitial deposits in addition to glomerular and arteriolar involvement. Moreover, exclusively, glomerular involvement does not appear to be entirely diagnostic of AFib since Oe et al. (311) recently reported AL kappa amyloid limited to the glomeruli in a pattern that was very similar to that seen in AFib. Extrarenal involvement is increasingly recognized, including systemic vascular, cardiac, gastrointestinal, splenic, autonomic, and peripheral neuropathy as well as abdominal fat involvement (512–514). Reactivity with commercial antibodies to fibrinogen is also variable and depends on the type of mutation (324).

Pathogenesis

Fibrinogen, produced exclusively by the liver (515), is a major component of the coagulation cascade. While various mutations in fibrinogen render it amyloidogenic, no abnormalities in coagulation have been observed. Moreover, in patients with AFib, plasma fibrinogen levels appear to be normal, except in homozygotes (513).

Despite the autosomal dominant mode of inheritance, owing to variability in penetrance, carriers of the mutation may be asymptomatic. Although initially considered to be predominantly nephropathic, AFib is emerging as a truly systemic amyloidosis with visceral, vascular, cardiac, and neurologic involvement (Fig. 22.25) (512–514). Hence, the question arises as to what is the primary target of the disease process. In view of the significant systemic vascular atheromatous involvement and the detection of the fibrinogen variant in vascular walls and in atheromatous plaques, linking AFib with atherosclerosis may be plausible. To this end, Stangou et al. suggested that direct amyloid deposition in vascular walls may be the first step in a disease process that leads to impaired endothelial function and that a subsequently developed nephrotic syndrome with hyperlipidemia and hypertension facilitates atheroma formation. If confirmed, these findings may alter our approach to the management of asymptomatic AFib carriers and nonscreened family members. Interestingly, several other proteins that are involved in vascular pathology and atherosclerosis, such as apolipoproteins I, II, and IV and the SAA protein, are also involved in amyloidogenesis (366,504,516).

Initially, treatment of patients with AFib was by kidney transplantation alone. However, solitary renal allografts fail within 1 to 7 years as a consequence of recurrent amyloidosis. Since fibrinogen is produced exclusively by the liver, liver transplantation combined with kidney transplantation was subsequently tried and shown to be curative (512,517). In contrast to ATTR, in AFib, post–liver transplantation, no deposition of the wild-type fibrinogen seems to take place.

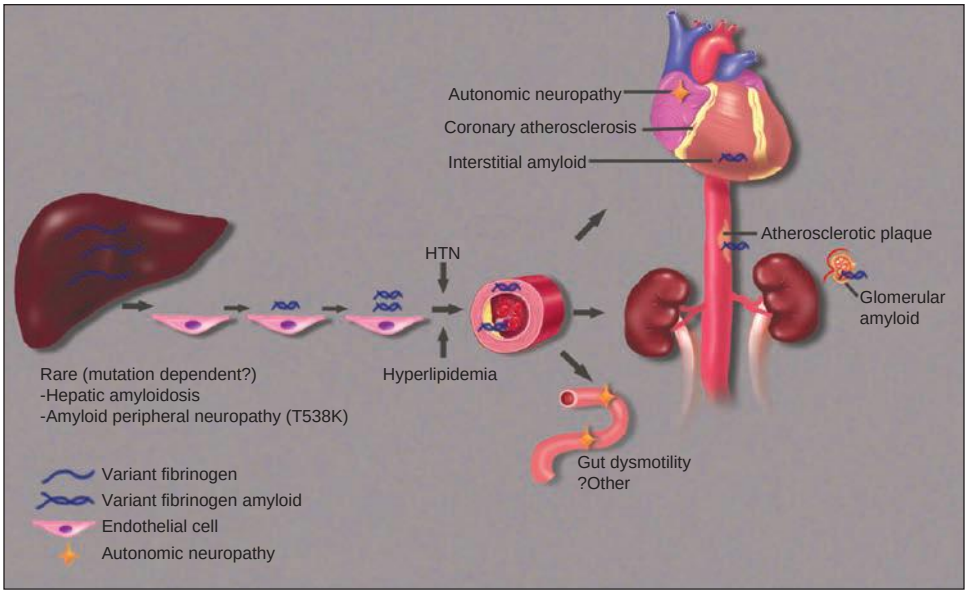

FIGURE 22.25 Proposed pathogenesis of fibrinogen amyloidosis. (From Picken MM. Fibrinogen amyloidosis: the clot thickens! *Blood* 2010;115:2985.)

Hence, a combined liver and kidney transplantation was favored over solitary kidney transplantation. Nonetheless, Stangou argued that AFib is a systemic and serious disorder that affects more organs than just the kidneys, and hence, the authors advocated early (or even preemptive) transplantation of the liver alone, before renal failure and significant cardiovascular amyloidosis develop, especially since the latter may preclude transplantation. The fact that explanted livers can be used for domino transplantation should also help alleviate organ shortage (512). While the discussion continues, it is becoming apparent that the diagnosis of amyloidosis may need to be accomplished early in the disease process, possibly even at a preclinical stage in asymptomatic carriers (512,514).

Miscellaneous Types of Hereditary Amyloidoses

Other types of familial systemic amyloidoses, including amyloid derived from apolipoprotein I (AApoAI) and apolipoprotein II (AApoAII) and amyloid derived from lysozyme (ALys), gelsolin (AGel), and cystatin (ACys), are relatively rare and are associated with amino acid substitutions in the corresponding native protein (366,516,518–525). More recently, a single case each of hereditary systemic amyloidosis derived from a mutant of SAA and β_2-microglobulin was discovered (please see also above and below in the corresponding segments). All of these amyloidoses can involve the kidney, and it is quite possible that additional types will be discovered in the future.

Of this group, apolipoprotein AI (ApoAI) is the most common systemic hereditary amyloidosis (366,504,516,518). ApoAI, synthesized in the liver and small intestine, is the major constituent of high-density lipoproteins in plasma and promotes the efflux of cholesterol from cells. At least 19 different mutations have been associated with this disease and patients identified in the United States, Spain, South Africa, Germany, Italy, and the United Kingdom. One reported variant was incidental in a patient with systemic AL amyloidosis (366). Interestingly, the phenotype of the disease varies based on the location of the gene mutation (366,504,518). Mutations in the amino terminus of ApoAI are associated with renal, hepatic, and, occasionally, cardiac amyloid deposition. In contrast, amino acid changes at the carboxyl terminus cause laryngeal, cutaneous, and cardiac amyloid deposits. The absence of a family history is apparently unusual in AApoAI and might suggest de novo mutation (366,504,518).

There is a substantial phenotypic heterogeneity among patients with identical ApoAI variants, which implies that other genetic and environmental factors may influence the clinical manifestations.

While renal disease with slow progression to hypertension and renal failure without nephrotic syndrome is frequent, some patients may have extensive visceral deposits in the liver, spleen, and kidneys, with end-stage failure as young adults (366,504). In contrast, other patients may have deposits limited to the larynx and/or skin, which may be very indolent and clinically insignificant (366,504). Occasional involvement of the heart, nerves, larynx, and gastrointestinal tract has also been reported. The renal pathology is fairly characteristic, with amyloid deposits detectable in the interstitium and medulla, while the glomeruli are usually spared. Amyloid deposits may be small and limited to large arteries. Thus far (366), only a mutant protein has been detected in amyloid deposits, with noninvolvement of the wild-type apolipoprotein AI.

AApo AII is characterized by slowly progressing renal disease with glomerular, interstitial, and vascular involvement by amyloid (520). Interestingly, systemic amyloidosis, apparently derived from a wild apolipoprotein AIV, was also reported (see earlier in this section).

ALys is characterized by nephropathy, dermal petechiae, gastrointestinal involvement with bleeding, hepatic involvement, and ocular or oral sicca syndrome (504). In the kidney, there are glomerular and vascular deposits of amyloid (504,521,522). AGel is characterized by cranial neuropathy, corneal dystrophy, and cutis laxa (523,524). Involvement of the kidneys has also been documented in homozygous patients (523) with marked glomerular amyloid deposits. ACys is typified by involvement of the cerebral vessels, in the form of familial cerebral congophilic angiopathy. However, there are also systemic deposits of amyloid, including the kidneys, where the deposits are clinically silent (504,525).

Etiology and Pathogenesis

While the presence of mutations in the familial forms may confer amyloidogenicity, other factors (genetic, environmental, aging) may also contribute to the process of amyloidogenesis. Similar to ATTR, variations in phenotype have also been observed in these rarer hereditary amyloidoses (504). Interestingly, several proteins associated with lipid transport and atherogenesis have been involved in amyloid formation, both familial (ApoAI and ApoA II) as well as apparently sporadic forms (ApoAIV and SAA). The detection of wild-type amyloid suggests that apolipoproteins may share structural features that render them amyloidogenic.

Dialysis-Related Amyloidosis: Amyloidosis Derived From β_2-Microglobulin ($A\beta_2M$)

Historical Perspective

β_2-Microglobulin amyloidosis ($A\beta_2M$) is a type of systemic amyloidosis that commonly develops in patients with chronic renal failure who are undergoing long-term hemodialysis (377,378,526–531) and is referred to as dialysis-related amyloidosis. Although the clinical symptoms of $A\beta_2M$ were first observed in the mid-1970s, the amyloid fibril protein was not identified until 1985 (377,378). The amyloid fibril precursor protein is β_2-microglobulin, a small, 11,800-Da protein with a predominantly β-pleated sheet secondary structure. β_2-Microglobulin is a subunit of the class I histocompatibility antigens. It circulates unbound as a monomer, and 95% is eliminated by glomerular filtration, tubular resorption, and metabolism. Thus, the serum level is inversely related to the glomerular filtration rate. Normal serum concentration (1 to 3 mg/L) increases up to 60 times in patients receiving maintenance hemodialysis because this protein is not efficiently removed during dialysis and there is also increased production from chronic inflammatory stress associated with the hemodialysis itself.

Since its discovery, it has always been presumed that $A\beta_2M$ represents an acquired amyloidosis affecting only patients with chronic kidney disease treated with dialysis and associated with increased levels of the circulating precursor protein (526–531). However, recently, Valleix et al. (369) reported a kindred with autosomal dominant hereditary systemic amyloidosis caused by a β_2-microglobulin variant (Asp76Asn). These patients presented with slowly progressive gastrointestinal symptoms and

autonomic neuropathy. Interestingly, the affected members of this kindred had normal renal function and normal circulating β_2-microglobulin values.

Clinical Presentation and Laboratory Findings

The typical clinical presentation includes carpal tunnel syndrome, chronic osteoarthropathy, spondyloarthropathies (predominantly involving the cervical spine), subchondral bone cysts, and fractures (526,531–533). The symptoms of osteoarthropathy range from acute arthritis to progressive joint destruction (531–533). Extra-articular symptoms may include ischemic colitis with perforation, macroglossia, and heart failure (534–538).

Clinical manifestations of the disease are 0 at 5 years, but increase to 50% at 12 years (520). Most patients with visceral amyloid were on hemodialysis for 15 years or more. The incidence of amyloid in peritoneal dialysis is less well known, since very few patients remain for prolonged periods of time on this type of dialysis. However, since peritoneal dialysis is unable to remove β_2-microglobulin, the risk may be similar. Among patients on peritoneal dialysis >5 years, by serum amyloid P component scintigraphy, the prevalence of dialysis-related amyloidosis was similar to that seen in hemodialysis patients (539). However, the development of bone cysts required much longer periods of dialysis (17 years), and many patients were asymptomatic (526). The incidence of dialysis-associated amyloidosis in the United States, and worldwide, is not known, and it remains underdiagnosed (526).

Gross and Microscopic Pathology

$A\beta_2M$ has a predilection for the bones, joints, and synovium. Radiologic and pathologic studies demonstrate juxta-articular cysts at insertion sites of capsule or tendons, which are filled with collagen and $A\beta_2M$. These cysts may become large enough to cause pathologic fractures (526–531). Clinically, $A\beta_2M$ osteoarticular deposits are usually diagnosed based on clinical and radiologic findings, and confirmation by tissue diagnosis is rarely sought. Although, typically, $A\beta_2M$ is associated with arthropathies and a carpal tunnel syndrome, visceral involvement has been documented by both pre- and postmortem studies. Interestingly, the heart is frequently involved, followed by the gastrointestinal tract, lung, and spleen. Bulky deposits may lead to macroglossia and bowel infarction and/or perforation. Predominantly, vascular deposits may be seen in many other sites including the skin and subcutis, ears, liver, thyroid, and perineural space. In contrast to visceral organs, vascular amyloid deposits are rarely seen in the osteoarticular tissues. $A\beta_2M$ is deposited in end-stage kidneys, but this has no clinical significance. Electron microscopic studies suggest that in some cases the β_2M amyloid fibrils are arranged in short curvilinear bundles (377,378).

Tissue deposition occurs much earlier than any clinical or radiographic manifestations of the disease. Hence, surveillance, in particular in high-risk patients, may be helpful in early detection of the disease and implementation of measures preventing or delaying disease progression before the development of irreversible debilitating complications. At autopsy, amyloid was found in joints in 21% of patients on hemodialysis for <2 years, 50% at 4 to 7 years, and 100% at more than 13 years (538). Interestingly, nonfibrillar deposits of β_2M have also been demonstrated in the heart and the spleen.

Etiology and Pathogenesis

Although there is no direct correlation between the absolute concentration of β_2-microglobulin and amyloidosis-related symptoms, high serum levels of this protein are believed to be the basis of amyloid deposition in tissues (526–531). Interestingly, however, no significant accumulation of β_2-microglobulin is observed in the synovial fluid, even though periarticular tissues are preferentially involved in this type of amyloidosis. Therefore, the involvement of factors modifying β_2-microglobulin has been postulated. To this end, it has been proposed, but not as yet conclusively proven, that modification of β_2-microglobulin by advanced glycation end products may facilitate amyloid formation (540–542). It has also been hypothesized that chronic inflammatory stress, induced by repeated stimuli from dialysis systems, may be involved in the pathogenesis of $A\beta_2M$. The role of genetics in $A\beta_2M$ has emerged recently with the identification of a hereditary form. The recently discovered β_2-microglobulin variant (Asp76Asn) was shown to be thermodynamically unstable and remarkably fibrillogenic in vitro under physiologic conditions (369).

Advanced age is a known risk factor, and amyloidosis is seen earlier in older patients than it is in younger patients with a similar duration of dialysis. Similarly, longer duration of dialysis has been associated with an increased incidence of orthopedic complications.

Interestingly, in recent years, there has been a decrease in the prevalence of $A\beta_2M$ (526). This decrease has been observed in the absence of any major modification in serum levels of β_2-microglobulin. However, it has been noted that this decline coincides with improvements in dialysis techniques, such as the use of endotoxin-free dialysates, which leads to better control of the inflammatory reaction associated with dialysis (526).

Treatment, Course of the Disease Process, and Prognosis

The relative efficiency of different forms of dialysis in removing β_2M from the circulation and the bioreactivity of certain dialysis membranes have been intensively studied in an effort to prevent $A\beta_2M$ (526–530). However, thus far, renal transplantation remains the only effective method of preventing and treating dialysis-related amyloidosis (526). Renal transplantation generally arrests the disease process and leads to rapid relief of osteoarticular pain. Scintigraphy studies also suggest that there may be some regression of the amyloid deposits (526,531). The future development of β_2M fibrillogenesis inhibitors may be another promising treatment strategy.

Amyloid Deposits in the Genitourinary Tract Outside the Kidneys

Extrarenal amyloidosis, involving other segments of the genitourinary tract, is not commonly reported. When it occurs, the amyloid deposits may be a part of a systemic or localized process. In systemic amyloidoses, nonrenal deposits are usually either clinically silent or less prominent. However, testicular amyloidosis leading to infertility is increasingly recognized. Systemic amyloidoses frequently involve the endocrine system, and endocrine dysfunction is increasingly recognized (482,483) including that of the thyroid gland, testicular amyloid deposits, and adrenal dysfunction. Testicular involvement is not uncommon in the systemic amyloidoses, in particular in AA, AL, AApolipo AI amyloidosis, ATTR, and dialysis-associated

amyloidosis (483). Testicular involvement is frequently seen in systemic AA and AApolipo AI amyloidosis, in particular, and since it may affect young adults, its clinical relevance is increasing (483,484). Testicular involvement in AA, AL, and AApolipo AI may be associated with testicular enlargement and lead to abnormal spermatogenesis and secondary infertility (483,484). In patients with known amyloidosis or identifiable risk factors (e.g., FMF), sperm cryopreservation and early sperm retrieval may be considered. While a more detailed description is beyond the scope of this chapter, the reader is advised to consult the corresponding reference (543).

Localized Amyloidoses of the Genitourinary Tract

Localized amyloid may form a single amyloid mass, referred to as an amyloid tumor or amyloidoma, which clinically may mimic neoplasia (543–549). Localized amyloid may be seen in the respiratory, gastrointestinal, and genitourinary tracts, skin, soft tissues, conjunctiva, lymph nodes, and elsewhere in individuals who do not have systemic amyloidosis (383). In the urinary tract, the most common localized deposits of amyloid are in the urinary bladder, but the ureter, urethra, renal pelvis, glans penis, prostate gland, and seminal vesicles as well as retroperitoneum may also be involved (543–552).

While localized genitourinary amyloid is most often of the AL type, other types of amyloid have also been detected: ATTR, AA, and, in patients on hemodialysis, $A\beta_2M$ (543). Amyloid may be detected also in urinary cytology (553,554). In localized AL deposits, there is frequently infiltration of plasma cells in the vicinity of the deposits. Immunohistochemical and molecular studies have shown that the plasma cell populations associated with local amyloid deposits are clonal (383), which suggests local production of the amyloid precursor protein. It is postulated that, in other types of localized deposits, local tissue factors may create a milieu favorable for fibrillogenesis (383). The treatment is local and involves excision of the lesion or radiation; some patients receive no treatment (543). The most critical aspect is correct determination of the localized nature of the deposits.

Systemic Amyloidoses: Treatment, Course of the Disease Process, and Prognosis

This section will discuss the systemic amyloidoses. The localized and dialysis-related amyloidoses are discussed in their respective sections; certain aspects pertaining to treatment strategies for hereditary/familial amyloidoses were also included in the discussions of their pathogenesis earlier in this chapter. From a patient treatment point of view, the diagnosis of renal amyloidosis should be considered to comprise three broad categories: AL, AA, and hereditary types. The treatment of patients with systemic AL targets the underlying plasma cell dyscrasia, while in patients with AA, control of the acute-phase response is the ultimate goal of therapy. In several hereditary amyloidoses, liver transplantation is performed to eliminate the source of abnormal protein, but pharmacologic therapies are also being currently tested.

In general, although the overall prognosis in untreated amyloidosis is poor, it varies with the type of amyloidosis and extent of clinical organ involvement. The last two decades have been associated with major advances in treatment of systemic amyloidoses (406,423,475,555–560). In general, in *all* types of systemic amyloidoses, the survival has been shown to improve with *early* treatment. However, the prognosis also depends on the extent of systemic involvement. While cardiac involvement and progressive neuropathy may dominate the clinical picture in AL as well as several hereditary amyloidoses (555–560), the involvement of the kidney, liver, and gastrointestinal tract may also be a cause of significant morbidity in several systemic amyloidoses (316,504,560). Thus, once a diagnosis of the type of amyloidosis is confirmed, it is important to assess the extent of systemic involvement and the prognosis. Renal amyloidosis is virtually always associated with a systemic amyloidosis. Traditionally, AL has been associated with the worst prognosis. Although long-term survival of patients with AL amyloidosis (more than 20 years) has been documented (561), such indolent progression of the disease is very uncommon. Untreated patients with AL amyloidosis have a median survival of 10 to 14 months from diagnosis (562). Before the introduction of high-dose melphalan and stem cell transplantation, fewer than 5% of patients survived >10 years (563). However, introduction of treatment of AL amyloidosis with high-dose melphalan and autologous blood stem cell transplantation in the 1990s has led to a marked improvement in overall survival, and currently, rates of overall median survival of between 5 and 6.3 years are being reported (564,565). In one recently reported series, 43% of patients survived >10 years (555); similar results have recently been reported by other major amyloidosis treatment centers (556–558,564). Nevertheless, despite these milestone advances of the last decade, 30% of patients still die within a year of diagnosis. Late diagnosis, after advanced organ dysfunction occurred, remains a major impediment to improving outcomes (565,566). The greatest pretreatment prognostic value is associated with the number of organs involved. Among the latter, cardiac involvement is the most important negative predictor of survival in AL (406,560,562,563). Cardiac involvement is seen in 60% of patients with AL. Death occurs as a result of congestive heart failure and/or arrhythmia. While cardiac infiltration by amyloid deposits has been traditionally considered to be the cause of heart failure, in recent years, the direct myocardial toxicity of the circulating paraprotein has been realized. Thus, currently, diagnosis of cardiac AL calls for a rapid suppression of the circulating monoclonal light chain amyloidosis. When severe cardiomyopathy precludes treatment with intensive chemotherapy, heart transplantation may be considered. In AA amyloidosis, cardiac involvement is relatively rare, but, when it occurs, it is also an unfavorable predictive factor (422). In hereditary ATTR amyloidosis, survival depends on the type of mutation, but in general a decline in cardiac function is less precipitous than in AL (504). Ultimately, however, heart failure develops, and heart transplantation may be considered. In patients with senile ATTR, the prognosis is better, typically with a 5-year survival generally achieved (507).

The kidney is one of the most common sites for amyloid deposition in systemic amyloidoses. Renal involvement is seen in 70% of AL patients and in 97% of patients with AA amyloidosis. Clinically relevant renal disease in hereditary amyloidoses depends on the amyloid type and the mutation (278,406,567,568). Not only renal dysfunction, which frequently progresses to end-stage renal disease, is the most common clinical manifestation of AL, but the diagnosis of the disease is also most commonly obtained by kidney biopsy. In AL amyloidosis, kidney involvement also affects prognosis adversely, but not to the same extent as cardiac involvement.

However, when corrected for cardiac involvement, serum creatinine level was an independent predictor of overall survival in one recent study (567). Nephrotic range, frequently massive proteinuria, and refractory peripheral edema are the predominant clinical manifestations in these patients. As the disease progresses, proteinuria usually becomes more marked, and a progressive decrease in renal function typically occurs. Among patients presenting with renal AL, 42% ultimately received renal replacement therapy. Presenting 24-hour urine protein loss and creatinine values predict which patients will require dialysis. Median survival for patients starting dialysis is <1 year. The presence of λ light chain amyloid predicts the increased likelihood of renal involvement (567).

Hematopoietic cell transplantation can reverse amyloid deposition and reduce or eliminate the clonal plasma cell disorder (555–560). Performance status and quality of life of patients with AL amyloidosis can be improved considerably (566). The main drawback is that treatment-related mortality of hematopoietic cell transplantation is relatively high ranging from 11% overall and recently decreased to 6% in the last 5 years (566). Experimentally, mesangial stem cells have been shown to be able to repair the damaged mesangium (409).

Several studies, using high-dose chemotherapy and autologous stem cell transplantation, have demonstrated clinical benefit in patients with AL amyloidosis, including improvement of renal function (556). Severity of baseline proteinuria predicts renal response in AL (569).

In AL, renal outcome (and overall outcome) is strongly associated with response to therapy as measured by serum FLC response. Thus, renal (and overall) outcome is best among patients achieving 90% suppression of the paraprotein. Again, early diagnosis and early treatment are critical. Clinical organ response is assessed according to established consensus criteria (570).

In AA amyloidosis, the clinical picture is frequently determined by renal involvement (278). In a recent large study from a single referral center in the United Kingdom, among patients not on dialysis, 97% had significant proteinuria, and 58% were nephritic (278). In older series (451), nephrotic AA patients had a life expectancy similar to that of AL. However, recent studies show that effective control of the underlying inflammatory activity, as assessed by median SAA concentration, is associated with improved survival (278,422,423). However, renal insufficiency at the time of diagnosis adversely influences the clinical outcome. Thus, early diagnosis is critical.

In AA amyloidosis, control of the acute-phase response is currently the standard of care (278,422,423). DMARDs, alkylating agents, anti–tumor necrosis factor (TNF) therapies, and other biologic agents have been used to treat inflammatory arthritides. Antibiotics and surgery have been used in the control of chronic sepsis, while corticosteroids, anti-TNF therapies, and surgery have been recommended for the control of Crohn disease. In Castleman disease, surgery and anti–IL-6 therapies showed success. Among familial periodic fevers, treatment regimens vary. FMF has been successfully treated with colchicine (426). Anti-TNF therapies and corticosteroids have been used in the treatment of TRAPS (tumor necrosis factor receptor–associated periodic syndrome), while in Muckle-Wells syndrome (member of CAPS), anti–IL-1 therapies have been tried (423). New pharmacologic therapies have also been explored (422,423,475). The first of a new class of antiamyloid agents is designed to block the formation of amyloid fibrils by inhibition of GAG binding (475) (see also under pathogenesis of AA).

Although most of the hereditary amyloidoses affect the kidney, there is significant variability in the degree of renal involvement. AFib leads to rapid renal failure (305,316,504,511,512), whereas renal failure in AApo AI and II and Alys is slower (504). There is also considerable variability in the renal involvement associated with various mutations in ATTR. Thus, while some TTR mutations are associated with renal failure (316,513), others may be associated with clinically silent renal deposits. Clinically relevant renal involvement is associated with homozygous AGel amyloidosis.

In hereditary amyloidoses, most of the abnormal precursor proteins are produced by the liver. Hence, liver transplantation is currently offered to many of the affected patients in an attempt to eliminate the input of an abnormal amyloid precursor protein, and a website tracking this activity is maintained at http://www.fapwtr.org (571). In several patients, liver transplantation was combined with cardiac and/or kidney transplantation (512,513,572,573). While the initial results have been encouraging, more recent data show that the effect of liver transplantation is also dependent on the TTR mutation. Thus, in certain mutations, there is a progression of neuropathy and/or cardiomyopathy post–liver transplantation due to continued deposition of amyloid derived from wild-type TTR (573). Pharmacologic therapies for ATTR are also currently in clinical trials ((510), please see also earlier in this chapter).

As discussed earlier in this chapter, in AFib, liver transplantation may be curative (512).

Renal Transplantation in Amyloidoses

In AL amyloidosis, renal transplantation is optimally performed after production of the amyloidogenic light chain has been eliminated. Renal transplantation for systemic AL amyloidosis has a relatively good outcome, with 1- and 5-year patient survival of 95% and 67% and median graft survivals of 5.8 years (574–576). However, the outcome is influenced by the fibril protein precursor supply (574). Still, better outcomes have been observed in systemic AA amyloidosis, with 92% and 89% 5-year patient and graft survival, respectively (576,577). In AA amyloidosis, successful control of the acute-phase response is essential.

Renal transplantation in patients with hereditary neuropathic amyloidoses has typically been performed in combination with orthotopic liver transplant (571–573). However, in patients with hereditary amyloidoses predominantly affecting the kidneys, solitary kidney transplantation has been tried (512,513) but with mixed results. In patients with AFib amyloidosis, treated with a solitary renal transplant, there was a high recurrence rate of amyloid in the allograft (512). Thus, currently, hepatorenal transplantation is offered to these patients with an excellent outcome (512). In contrast, in apolipoprotein AII amyloidosis, where renal failure develops slowly, solitary kidney transplantation has been successful in at least one case (504) where a 9-year follow-up showed no allograft dysfunction.

Cardiac amyloidosis is a contraindication for renal transplant in AL and AA amyloidoses, because survival is markedly compromised (475). However, combined cardiac and renal transplantations have been offered to selected patients with

hereditary amyloidoses targeting those organs (475). While systemic complications are responsible for a significant percentage of posttransplant morbidity and mortality, kidney transplantation can be successfully performed in patients with AL who achieved complete hematologic response (574–576). Therefore, renal transplantation may be appropriate in some AL amyloidosis cases where the underlying plasma cell dyscrasia has been controlled, heart involvement is absent, and the patient is in relatively good shape. The great majority of transplants have been performed in patients with AA amyloidosis, which has a slower and more indolent clinical course in patients where the acute-phase response has been successfully controlled (577). Renal transplantation continues to be offered to patients with other amyloidoses, who have severe kidney amyloid deposition (572–577). In contrast to AL and AA, in hereditary amyloidoses, cardiac involvement is not an absolute contraindication to renal transplantation (573).

De novo amyloidosis in transplanted kidneys and recurrences in patients with AL and non-AL amyloidosis have also been reported (577–580). In one large series of 59 renal recipients with AA amyloidosis, the recurrence rate of AA amyloidosis nephropathy was estimated at 14%. As more experience is accumulated, a better definition of the role of renal transplantation in these diseases will emerge.

Differential Diagnosis for Amyloidosis

The diagnosis of amyloid can be made with certainty in the great majority of cases by using a combined approach including light microscopic, histochemical, and ultrastructural analysis (293–296,324–328,334,372). It is as important to identify the precursor protein using ancillary diagnostic techniques, with immunofluorescence and immunohistochemistry playing crucial roles in this process, so that the amyloid can be properly classified for patient management and therapeutic purposes (324–328). Introduction of proteomics and molecular methods have improved the specificity (334).

To diagnose early amyloidosis, a high level of suspicion is needed. Congo red and thioflavin T or S stains may not clearly identify small foci of amyloid deposition, causing the diagnosis to be missed. Ultrastructural evaluation, if representative material is available, is often the best way to substantiate the diagnosis of amyloidosis in these cases. Segmental glomerular and vascular hyalinosis may at times be confused with amyloidosis because of the similar eosinophilic (hyaline) light microscopic appearance.

Other infiltrative glomerular processes must be ruled out. Fibrillary and immunotactoid glomerulopathies may be associated with expanded mesangium and loss of argyrophilia, features that can also be seen in amyloidosis. The negative Congo red stain in the above conditions and ultrastructural findings should readily differentiate these diseases (372). The reader is referred to the corresponding chapter on these entities for a full description of the differential diagnostic points. Nodular glomerular amyloidosis can be confused with other nodular glomerulopathies such as diabetic nephropathy and LHCDD; the tinctorial characteristics of amyloid, as described under the light microscopy section of this chapter, and ultrastructural features should provide enough evidence to make the right diagnosis.

Although the ultrastructural appearance of amyloid is unique, a source of confusion with amyloid fibrils may be mesangial matrix with an accentuated fibrillary appearance (i.e., mesangiolysis and diabetic fibrillosis) (373–376). The random distribution and rather constant diameter of amyloid fibrils should provide the necessary criteria for making a distinction in these situations.

RENAL INVOLVEMENT IN WALDENSTRÖM MACROGLOBULINEMIA

Historical Perspective

WM was originally described by Jan Waldenström in 1944 (581). He reported two elderly patients with "signs of severe derangement of protein metabolism with very high globulin and low albumin values." These patients also had a severe normochromic anemia, high serum viscosity, and a very large molecule with a molecular weight of 1,000,000 in the serum, which migrated next to β-globulin (581). Dutcher and Fahey (582) detailed the histopathologic and clinical features of WM in 1959 and described perivascular infiltrates of neoplastic lymphoplasmacytic cells in the kidneys in one of three autopsies that they reported.

Several case reports in the 1940s and 1950s noted an association between WM and amyloidosis. It was Lamm in 1961 who called attention to peculiar renal alterations in patients with WM. He described hyaline thrombi in glomerular capillaries and in an interlobar band of the renal artery in one of two autopsies of patients with WM (583). In 1966, Forget et al. (584) reported one case and summarized the literature. Although the pathogenesis was unclear at the time, their paper served to affirm that a small percentage of patients with WM during their clinical course develop amyloidosis. Argani and Kipkie coined the term *macroglobulinemic nephropathy* in 1969 (585). A comprehensive publication in 1970 by Morel-Maroger et al. (586) reviewed findings of 5 renal biopsies and 13 autopsies of patients with WM. In 2008, Audard et al. (587) reported 14 patients with a circulating monoclonal IgM and kidney disease and revisited the disease spectrum.

Clinical Presentation and Laboratory Findings

WM is a rare disease; it is much less common than myeloma. It is far more common in whites than in blacks, and it is more frequent in female whites, with an incidence of 6.1 cases per million individuals (588). It is a lymphoproliferative disorder characterized by a monoclonal proliferation of lymphoplasmacytic cells producing IgM, which can be detected as an M spike in SPEP. It generally occurs in older patients (median age of 63 to 70 years), and 99% of the patients are older than 40 years (589). IgM is the paraprotein found in 16% of all myeloma patients, but only 17% to 26% of these patients develop WM (587). IgM monoclonal gammopathy may exist for years before WM develops (590). A consensus panel has made recommendations regarding the clinicopathologic definition of WM (591), as the definition of this syndrome has been controversial (592).

The diagnosis of WM is sometimes made in the course of workup for anemia, elevated sedimentation rate, and/or increased serum proteins (589,592–594). Patients may also present with a variety of clinical signs and manifestations,

including fatigue, weakness, recurring bleeding, lymphadenopathy, splenomegaly, anemia, elevated erythrocyte sedimentation rate, high serum viscosity, and decreased fibrinogen (584,592–594). The serum level of IgM is variable, but symptomatology generally occurs when the serum IgM levels exceed 3 g/dL, and hyperviscosity occurs as a consequence of the high molecular weight of IgM. Monoclonal IgM proteins have very high molecular weights, and when they accumulate, increased plasma osmotic pressure and plasma volume follow. In about one third of patients with WM, a hyperviscosity syndrome occurs (594), characterized by chronic nasal, gum, and gastrointestinal bleeding; headaches; tinnitus; vertigo; impaired hearing; ataxia; mental confusion; blurring and loss of vision with sausage-shaped retinal veins; flame-shaped hemorrhages in the optic fundus; and papilledema (594). Finally, high-output heart failure, stupor, and coma may occur. Osteolytic lesions are rare (595). Cryoglobulinemia may be an associated condition (588). Unusual manifestations occur in rare cases, such as multifocal osteolytic lesions, bone fractures, and those related to infiltration by neoplastic plasma cells of multiple sites, producing such clinical manifestations as cardiac tamponade, obstruction of bile ducts, and severe headache, lethargy, and stupor (596).

In contrast with classic plasma cell dyscrasia and myeloma, renal abnormalities occur rather infrequently in WM (587). Glomerular abnormalities predominate (586). Renal function is mildly or moderately impaired in about 15% of patients with this condition (586). Patients may present uremic and dehydrated and exhibit nonselective proteinuria (597). Rarely, a patient with WM presents in acute renal failure (585). Patients with WM are typically not hypercalcemic. The degree of BJ proteinuria is low, explaining why cast nephropathy is so rare in this condition. While Krajny and Pruzanski (598) found that BJ proteinuria was identified in 71% of 45 patients, the light chain excretion was very low (<200 mg/24 h in all but 9 patients). Kyle and Garton reported a monoclonal spike in the urine in 25 of 57 patients with a median excretion of 1 g/24 h (445). Occasional patients develop nephrotic or nephritic syndrome. Eleven of these patients also had albuminuria (589,599). Infiltration of the renal parenchyma by neoplastic cells can also occur (600,601). Amyloidosis develops in 20% of patients with WM; when this happens, the kidneys are often affected. Amyloidosis is the most common cause of nephrotic syndrome in these patients. Significant albuminuria is usually related to amyloidosis (602,603).

Acute renal failure in patients with WM is usually a result of extensive vascular occlusion by circulating IgM, dehydration (586,597), massive renal infiltration by neoplastic lymphoplasmacytic cells (600), and distal nephron obstruction, as seen in myeloma (600,604). In addition, there is a case report of acute tubular necrosis in a patient with WM attributed to hyperviscosity (605).

Gross Pathology

Not much is known about the gross appearance of kidneys in patients with WM. There is one report of giant kidneys found in this condition in a patient with massive infiltration of the renal parenchyma by neoplastic cells (600).

Light Microscopy

The most typical glomerular lesions are variably sized, sometimes massive, amorphous, PAS-positive subendothelial deposits (Fig. 22.26A), which may result in compromise of the capillary spaces. In some cases, well-defined thrombi occlude the capillary spaces. Either a few thrombi or massive thrombosis can be seen (585,586). Glomerular necrosis, which may be segmental, is rare and is a result of complete occlusion of glomerular capillaries or arterioles. Cryoglobulins may be identified in 7% to 20% of these patients, and the thrombi may contain cryoglobulins (86,606,607). Therefore, there is significant morphologic overlap between patients with WM and those with cryoglobulinemia associated with other disorders. WM was identified in 7 of 21 patients with type I cryoglobulinemia and 8 of 22 patients with type II cryoglobulinemia, attesting to the close association between these two diseases and the morphologic overlap that may occur (606). Amyloid deposition can be seen in any of the renal compartments (608).

Other renal lesions have been reported in patients with WM, including minimal change disease (609), membranous

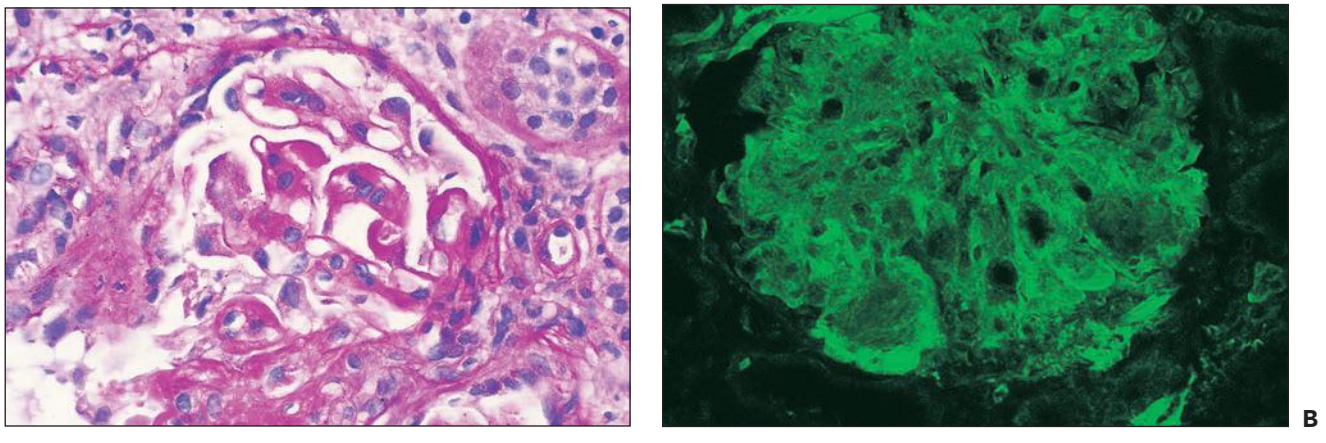

FIGURE 22.26 Waldenström macroglobulinemia. A: Typical massive subendothelial deposits, which are PAS positive. **B:** Corresponding fluorescence staining for IgM depicts peripheral capillary wall staining in areas where subendothelial deposits are present. (×500.)

nephropathy (610), immunotactoid and fibrillary glomerulopathy (610), crescentic glomerulonephritis (611), and MIDD (612). Cast nephropathy is unusual but has also been documented (504), as well as Fanconi syndrome; the latter attributed to a monoclonal FLC in a patient with WM (613).

Infiltration of the renal parenchyma by neoplastic cells represents an additional pathologic manifestation of WM. These infiltrating neoplastic cells are seen in both cortical and medullary areas and may play a crucial role in the majority of WM patients who develop significant renal insufficiency. In some cases, the neoplastic cellular infiltration results in the formation of renal or perirenal masses (614).

Immunofluorescence

The glomerular deposits vary in their distribution, stain for IgM and often concomitantly for IgG, and appear as granular to massive peripheral capillary wall deposits (see Fig. 22.26B). In some cases, the deposits are accompanied by C3 or C4 staining along the peripheral capillary walls. Light chain restriction, indicating monoclonality, may be identified in the distribution of the Ig deposits (443). In cases with monoclonal IgM, the light-chain isotype is usually kappa. If thrombi are present, they also frequently stain for IgM, with their peripheral zones generally staining more intensely than their centers (586,597–600). Even in normal-appearing glomeruli, there may be deposition of IgM. Arterioles and small arteries may also reveal IgM staining in their walls. Some of the staining may be related to the fact that these patients have large amounts of circulating IgM, which adheres to various structures in the kidney.

Electron Microscopy

The deposits are usually electron dense and are located subendothelially, in capillary spaces in the glomeruli, or in the extraglomerular vasculature. Most of the capillary thrombi are electron dense and amorphous (612), but some may contain cryoglobulins and exhibit focally organized substructure. Cryoglobulins may also be seen in thrombi located anywhere in the renal vasculature.

Treatment, Course of the Disease Process, and Prognosis

There are a number of features in this condition at presentation that adversely affect survival: male sex, age >60 years, anemia, and neutropenia. Some patients with monoclonal IgM in their serum evolve over years to develop WM (589,590). Treatment may not be needed until years after the diagnosis of WM is established; treatment is usually required because of hematologic complications such as severe anemia, hyperviscosity, or visceral involvement.

The incidence of renal manifestations associated with WM has declined as a result of improved treatment with control of levels of circulating IgM. Renal insufficiency is common in patients who are biopsied. Renal manifestations rarely require dialysis (585,597). In the small percentage of patients with hyperviscosity and renal failure characterized by vascular occlusion on renal biopsy by circulating IgM, plasma exchange may be very useful. In some patients, repeated plasmapheresis is employed to delay the use of cytotoxic drugs. However, in most patients, plasmapheresis alone is not enough to reverse the renal insufficiency, and the concomitant use of chemotherapy to suppress the underlying neoplastic process is indicated. Oral alkylating agents are employed most often, with chlorambucil administration on a daily basis at low dose or intermittently at higher doses. Approximately 50% of the patients achieve a sustained partial response (615). Steroids may be added to the treatment, especially if there is associated cryoglobulinemia or autoimmune hemolytic anemia.

In essence, the treatment of patients with WM and renal compromise consists of systemic chemotherapy to reduce tumor load and the use of plasmapheresis to remove pathologic circulating IgM. When cryoglobulinemia accompanies WM, proteinuria and renal function may improve with primary therapy of the condition, such as is seen when interferon-α therapy is used for the treatment of patients with hepatitis C and WM (607,610,616). The median survival for all patients is 5 years and, as expected, is better with responders to the therapy (593). When amyloidosis complicates WM, the overall survival is 28 months; this is even shorter for those patients with cardiac involvement at presentation (602). The most common cause of death in WM is gastrointestinal hemorrhage (589). A small percentage (3.8% to 7.4%) of these patients die of renal failure (589,600,617).

Transplantation in Waldenström Macroglobulinemia

There is minimal experience with renal transplantation in patients with WM. A renal transplant was performed in a patient who died 7 months later of a pulmonary embolus. Postmortem examination established a diagnosis of WM with renal involvement and served to retrospectively confirm a diagnosis of WM in a previous renal biopsy performed on this patient, diagnosed initially as chronic pyelonephritis (618).

Differential Diagnosis for Waldenström Macroglobulinemia–Associated Nephropathy

The histopathologic findings in WM-associated glomerulopathy overlap significantly with those seen in cryoglobulinemic nephropathy. Hyaline thrombi are characteristic of both conditions, and proliferative and exudative glomerular activity can be seen in selected WM-associated glomerulopathy cases, although it is more frequently seen in cryoglobulinemic nephropathy. Cryoglobulins can be identified in WM-associated nephropathy, further complicating the differential diagnosis between these two conditions (607). Therefore, the clinical history and laboratory data become very important when this differential diagnosis is considered. Fortunately, in the great majority of the cases, the renal biopsy is performed after a diagnosis of WM has been established, and the role of the renal pathologist is to determine the type of lesion present and the extent of the abnormalities encountered.

However, it should be noted that in a significant number of cases, the light microscopic and immunofluorescence findings clearly point to a diagnosis of WM-associated glomerulopathy. Identifying by immunofluorescence glomerular deposits or capillary thrombi that stain dominantly or codominantly for IgM with associated light chain restriction in the proper clinical setting should immediately suggest a diagnosis of WM-associated nephropathy (586).

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CHAPTER 23

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Glomerular Diseases With Organized Deposits

INTRODUCTION AND HISTORICAL BACKGROUND

The term glomerular diseases with organized deposits has come into use in the medical literature relatively recently with the gradual increase in number of glomerular diseases defined by recognition at the ultrastructural level of deposits with distinguishing organized substructures.

In the 1970s, consultation of a recently published textbook of renal pathology (1) would have produced only two such entities: amyloidosis and cryoglobulinemia. With the reporting of cases that were initially described as showing Congo red–negative amyloid-like glomerular deposits (2) and later named fibrillary deposits (3), the entity recognized by most renal pathologists as fibrillary glomerulonephritis (FGN) (4) came into existence. About the same time, the much rarer entity of immunotactoid glomerulopathy (ITG) was described (5). This was followed by the description of hereditary collagenofibrotic glomerulopathy (6) and fibronectin glomerulopathy (7). With the increase of these entities arose the need to have an all-encompassing term—hence, the emergence of the term glomerular diseases with organized deposits. The term was first used in *Heptinstall's Pathology of the Kidney* in its fifth edition, although the expression "with organized deposits" had been introduced in the renal pathology literature as early as 1967. This chapter focuses primarily on FGN and immunotactoid

glomerulopathy and also discusses other glomerular diseases with organized deposits that are covered in more detail in other chapters (e.g., cryoglobulinemic glomerulonephritis and collagenofibrotic glomerulopathy). In addition, normal extracellular materials that can be confused with organized deposits (e.g., fibrillary collagen and fibrin tactoids) are described. The organized deposits discussed in this chapter are the ones that are seen most often in renal biopsy specimens (8–10); however, there are anecdotal reports of organized deposits that do not fit well into these recognized categories (11).

As is the case with most renal biopsies, achieving the correct diagnosis is critical for management and prognostic purposes, but this is particularly true in the case of the topic under discussion where the difference, for example, between AL amyloidosis and FGN means the difference between a condition that can potentially be treated by appropriate chemotherapy or, if necessary, bone marrow ablation and autologous stem cell transplantation and another that currently can only be treated for the most part by measures directed toward control of proteinuria and hypertension.

Because many of the entities listed above are covered more extensively in other chapters of this text, this chapter focuses on FGN, immunotactoid glomerulopathy, fibronectin glomerulopathy, and the accentuation of the mesangial matrix and deposition of precollagen in some sclerosing conditions that enter into the differential diagnosis of in particular FGN. Glomerular diseases with organized deposits can be categorized in a number of ways.

Although for classification purposes it is useful to divide the entities that are the subject of this chapter into those that are Congo red reactive and those that are not (Table 23.1), from the practical point of view, this approach would be helpful only if the Congo red stain was included in the battery of special stains used on a routine basis to evaluate every renal biopsy just like the periodic Schiff (PAS) and the periodic methenamine silver (PAMS/Jones) stains are. However, if that is not the case, then an alternative diagnostic algorithm is needed that is based on the stains that are used by the majority of renal pathologists. That is why we find the algorithm based on silver methenamine staining outlined in Figure 23.1 helpful. An algorithmic approach is a useful first step in making a pathologic diagnosis, but the final diagnosis depends on considering multiple pathologic features that rule in or rule out a specific diagnosis (Table 23.2).

TABLE 23.1 Congo red reactivity of glomerular deposits with ultrastructural organized deposits

Congo red–positive
Amyloidosis composed of >30 precursor proteins among which are the following:
Immunoglobulin light chain (usually λ light chain but can be κ) (AL amyloid)
Immunoglobulin heavy chain (AH amyloid)
Immunoglobulin light and heavy chain (ALH amyloid)
Amyloid A protein (AA amyloid)
Apolipoprotein AI and AII
Apolipoprotein E
Transthyretin
Leukocyte chemotactic factor 2 amyloid
Fibrinogen Aα
Lysozyme
Gelsolin
Cystatin
β-Microglobulin (no renal involvement but complicates chronic hemodialysis)
Congo red–negative
Cryoglobulinemic glomerulopathy
Fibrillary glomerulonephritis
Immunotactoid glomerulopathy
Fibronectin glomerulopathy
Collagenofibrotic glomerulopathy
Diabetic fibrillosis and other sclerosis with accentuated collagenous fibrils
Fibrin

Glomerular deposits with ultrastructural organized deposits can be divided into Congo red–positive deposits of amyloid of varying composition versus Congo red–negative deposits of other substances.

FIBRILLARY GLOMERULONEPHRITIS

Clinical Presentation and Epidemiology

The first case of FGN was described in 1977 (2) in a patient who presented with the nephrotic syndrome and whose biopsy demonstrated immunoglobulin and complement deposits on immunofluorescence microscopy examination and on ultrastructural examination revealed the presence of fibrillary deposits resembling amyloid fibrils in a background of amorphous granular deposits consistent with immune complex–type deposits. All histochemical stains for amyloid were negative. This case was soon followed by a succession of publications that established this as a new diagnostic entity (reviewed in Ref. (12)). Most significant was the small series published by Duffy et al. (3), which used the title "fibrillary renal deposits and nephritis" and established the clinical presentation of the entity, as well as emphasized the need for electron microscopy to avoid missing this diagnosis. Credit for coining the term *fibrillary glomerulonephritis* goes to Alpers et al. (4). Multiple series have since been published that have further defined the clinical presentation, pathologic features, and prognosis of FGN, and the majority have in particular emphasized the distinction between FGN and the much rarer entity widely known as immunotactoid glomerulopathy (ITGP), although this latter issue was the subject of a heated debate for a number of years (12–19).

FGN is found in approximately 1% of native renal biopsies accessioned in most renal biopsy services (12,18). The clinical presentation is not specific. Patients are overwhelmingly Caucasians, even in series where this did not reflect the racial distribution of the overall biopsy population (12,20) with a slight female predominance. Two large series (18,19) give age means and ranges remarkably similar, approximately 57 [28 to 81] years and 53 [19 to 81] years, respectively; however, a case has been described in a 9-year-old girl (21), and we have seen a case in a 14-year-old boy. All patients have proteinuria at the time of biopsy with proteinuria often reaching the nephrotic level and frequently associated with the full nephrotic syndrome. Microscopic hematuria is present in approximately 50% to 60% of cases, and rare cases have episodes of gross hematuria (18,19). Renal insufficiency is

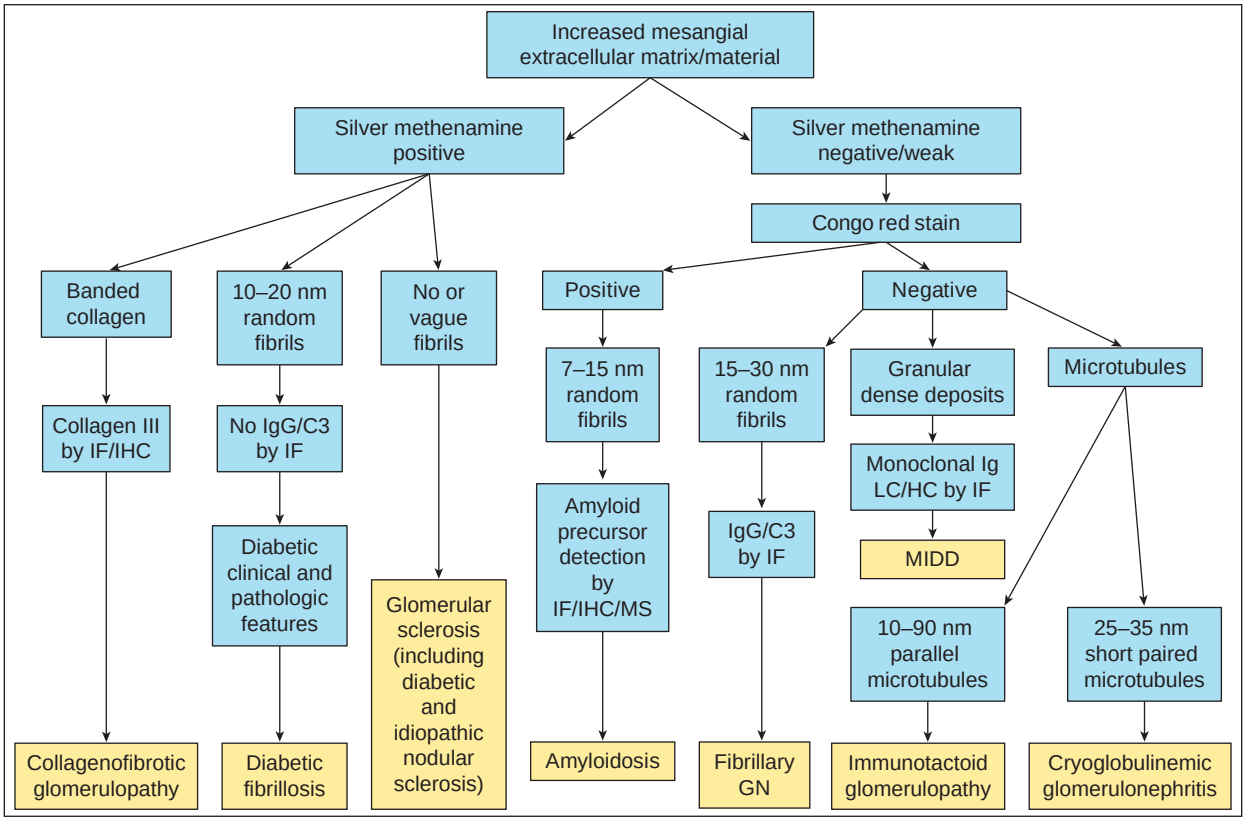


FIGURE 23.1 Diagnostic algorithm for glomerular mesangial extracellular matrix expansion based on silver methenamine staining. Many but not all processes with organized deposits are included. Rare entities such as fibronectin glomerulopathy and metabolic storage diseases are not included. MIDD, monoclonal immunoglobulin (Ig) deposition disease, including light chain (LC) and heavy chain (HC) deposition disease; GN,glomerulonephritis; IF, immunofluorescence microscopy; IHC, immunohistochemistry; MS, mass spectroscopy.

present in 50% of patients at the time of biopsy (16), and 65% are hypertensive (16). In the two most recent and largest series (18,19), associated medical conditions included, respectively, diabetes mellitus (20%, 20%), lymphoproliferative disease (2%, 9%), carcinomas (5%, 14%), and systemic lupus erythematosus (1.6%, 3%). In the series from the Mayo Clinic (19), an additional 15% had miscellaneous autoimmune diseases. Positive serologic tests in the same two series included hepatitis C (17%, 3%), monoclonal immunoglobulin by serum or urine protein electrophoresis (15%, 17%), antinuclear antibodies (16%, 14%), low complement level (2%, 2%), and cryoglobulins (4%, 3%). FGN is a disease confined to the kidney. There are individual case reports indicating involvement of the liver, lungs, and heart (22–24); however, careful examination of the published illustrations suggests to us as well as to others (25) that interpretation of these reports warrants caution. An autopsy study appears to have demonstrated the presence of the characteristic fibrils of FGN only in the spleen in addition to the kidney and confirmed that localization by the immunogold labeling method (26).

Pathologic Findings

Gross Pathology

Little is known about the gross pathology of FGN since patients reaching end-stage renal disease (ESRD) are kept alive on dialysis or receive renal transplants such that autopsy experience with the entity is limited. An occasional autopsy case on a patient who died of myocardial infarction close to 5 years after the diagnostic biopsy described atrophic right and left kidneys weighing 100 and 90 g, respectively, with coarse granular external surfaces (26).

Light Microscopy

The common denominator among all the studies including any significant number of cases (3,4,12,13,18,19) is the expansion of the mesangium by eosinophilic, PAS-reactive material (Fig. 23.2) with the exception of one study (18) where the material was described as weakly eosinophilic and weakly PAS reactive. With a Masson trichrome stain, the mesangium stains blue or green, depending on whether one uses an aniline blue– or light green–based stain, with a variable red fuchsinophilic tinge depending on the mixture of the collagenous mesangial matrix and deposits of pathologic material, respectively (Fig. 23.3). The periodic acid methenamine silver stain (PAMS or Jones stain) shows failure of the mesangium to stain uniformly with silver (Fig. 23.4). This should raise concerns about the potential diagnosis of amyloidosis; however, all histochemical stains for amyloid, specifically the most commonly used ones, namely, Congo red and thioflavin T, are negative. An important caveat is that in very rare instances, FGN is Thioflavin T positive.

TABLE 23.2 Distinguishing pathologic characteristics of different types of organized glomerular deposits

	Light microscopy	Immunofluorescence microscopy	Electron microscopy	Others
Amyloidosis	Faintly eosinophilic and PAS-negative material. Spicules best appreciated with silver stains with a porcupine (cock's comb) appearance distinguishable from spikes of membranous glomerulonephritis	Staining for specific subtypes of amyloid. Routine stains may show increased nonspecific background staining.	Feltwork of 7- to 15-nm randomly disposed fibrils	Apple green birefringence with polarized light. Fluorescence with Thioflavin T and S and Congo red
Cryoglobulinemia	Hyaline subendothelial and luminal material strongly PAS positive. MPGN-like pattern. Abundant monocytes	Staining for contributing immunoglobulins and light chain over or around hyaline thrombi	Curved 20- to 30-nm cylindrical and annular structures in subendothelium and lumen. Sometimes deposits amorphous	Circulating cryoglobulins, hypocomplementemia, and rheumatoid factor
Fibrillary glomerulonephritis	Mesangial expansion by eosinophilic and PAS-positive material. Other features highly variable	IgG and C3. Usually both kappa and lambda	Feltwork of randomly disposed 15- to 30-nm fibrils sometimes in background of amorphous immune complex–type material	Absence of congophilic material
Immunotactoid GP	Mesangial and capillary expansion by eosinophilic and PAS-positive material. Often pattern of membranous glomerulonephritis with characteristic spikes. Other cases MPGN-like pattern	IgG, often light chain restricted	Bundles of intersecting 10- to 40-nm microtubules, occasionally up to 90 nm. May be in mesangial, subendothelial, or subepithelial	Paraprotein often identified. Frequent association with lymphoproliferative malignancies
Collagenofibrotic GP	MPGN-like pattern with PAS- and PAMS-negative infiltrate	Usual immunofluorescence panel noncontributory	Frayed collagen bundles with 43- to 65-nm periodicity. Worm and comma shapes where bundles cut transversely	Identification of collagen III by immunofluorescence or other immunohistochemical methods
Fibronectin GP	Accentuation of lobular architecture. Strongly PAS-positive but PAMS-negative material	Usual immunofluorescence panel noncontributory. Positive staining for fibronectin	Some kindreds with 14- to 16-nm fibrils. Other kindreds show only amorphous deposits	Staining for fibronectin by IHC
Collagen deposits in various GPs	Histologic features are those of underlying condition, often with sclerosis	Immunofluorescence pattern of the underlying condition	Collagen recognized by characteristic periodicity and precollagen recognizable by its usual association with mature collagen	

Modified from Iskandar SS, Herrera GA. Glomerulopathies with organized deposits. *Semin Diagn Pathol* 2002;19(3):116–132.

The above-described mesangial pattern comprised 71% of cases in a large series reported from the Mayo Clinic (19); however, other patterns described (18) include membranoproliferative-like, diffuse proliferative and exudative, segmental necrotizing and crescentic, membranous and diffuse sclerosing patterns. Crescents are reported to be present in between 17% and 31% of cases (12,18–20) excluding the category of segmental necrotizing and crescentic glomerulonephritis, which was defined by Nasr et al. (19) as presence of ≥50% glomeruli with segmental tuft necrosis or crescents.

Immunofluorescence Microscopy

The great majority of cases have deposition of polyclonal IgG (Fig. 23.5) along with C3 and κ and λ light chains in a mesangial pattern as well as along the capillary walls. The staining is typically confined to the glomeruli, but we have observed one specimen with staining of arterioles (Fig. 23.5A). Staining for IgA, IgM, and C1q is variable. A small minority of cases stain monotypically for κ light chain (4). The capillary wall deposits can mimic membranous glomerulopathy, in some cases. In rare cases, the capillary wall staining appears linear

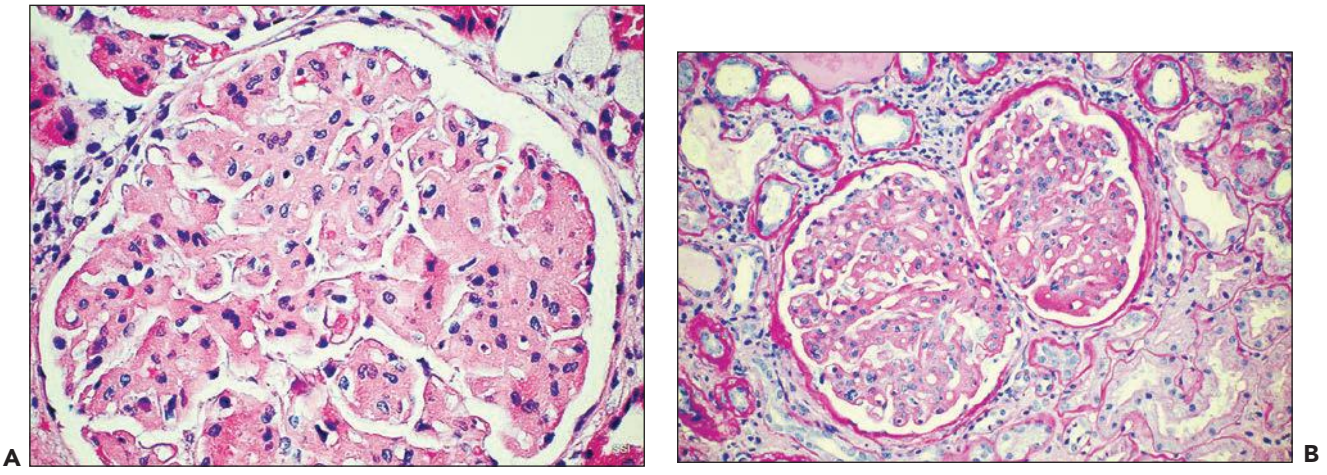


FIGURE 23.2 Fibrillary glomerulonephritis. A: Representative glomerulus from a case of FGN with extensive mesangial expansion. Note the infiltration of the mesangium by a homogeneous though slightly "moth-eaten" eosinophilic material. (H&E, 400×.) **B:** Case of FGN displaying a diffuse proliferative pattern and thickening of capillary walls. Note that the infiltrating material is PAS reactive. (PAS stain, 200×.)

and may simulate the appearance of anti–glomerular basement membrane autoantibody disease (27). The appearance of immunofluorescence in FGN is particular to the entity and allows one to anticipate the diagnosis in the great majority of cases even before electron microscopic examination, although of course by definition ultrastructural examination is required to render a confident diagnosis of FGN. Although the signal of staining for IgG is usually strong, it is neither granular nor linear and has been described as smudged (28) (Fig. 23.5). There is a clear predilection for staining with subclass IgG4 first demonstrated in 13 cases studied by Iskandar et al. (12) with only weaker staining in 15% of cases for subclass IgG1. This has generally been confirmed in a subsequent series (18). A single case of IgA-λ restriction has been reported (29). In two cases that appear to be bona fide FGN based on the published electron micrographs, attempts at immunofluorescence staining with antihuman immunoglobulin (Ig) antibodies from multiple commercial suppliers failed to demonstrate the presence of Ig deposits (30).

Electron Microscopy

FGN is largely defined by its ultrastructural appearance, summarized as infiltration of the mesangium and to a variable degree the glomerular basement membrane by randomly disposed fibrils that resemble amyloid fibrils but are about twice as thick and are less densely packed. Whereas the range of amyloid fibril thickness is 7 to 15 nm, that of fibrils in FGN is 15 to 30 nm with a mean of 22.2 nm and a standard

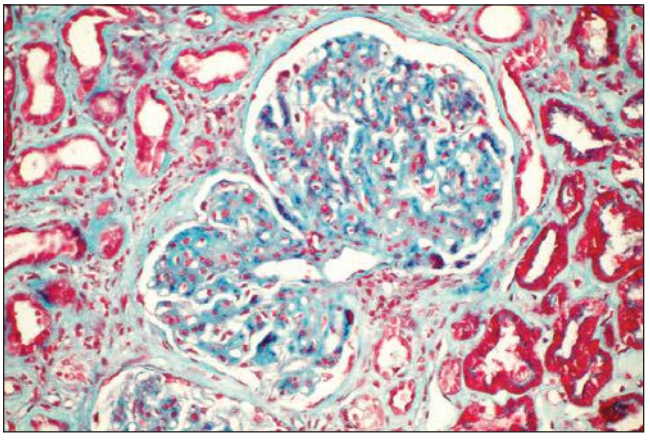


FIGURE 23.3 Fibrillary glomerulonephritis. Same case as in Figure 23.2 using Gomori trichrome green stain for collagen, showing the increased extracellular matrices staining green with various degrees of segmental fuchsinophilic tinge. (Gomori trichrome, 200×.)

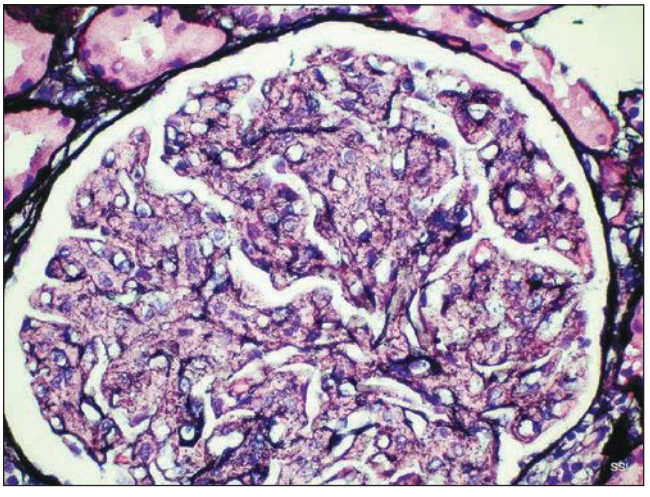


FIGURE 23.4 Fibrillary glomerulonephritis. Periodic acid-Schiff methenamine silver (PAMS/Jones) stain showing markedly reduced staining in the expanded mesangium and the thickened capillary walls. Staining is seen only in the residual GBM and remnants of fibrils of the mesangial matrix. (PAMS stain, 400×.)

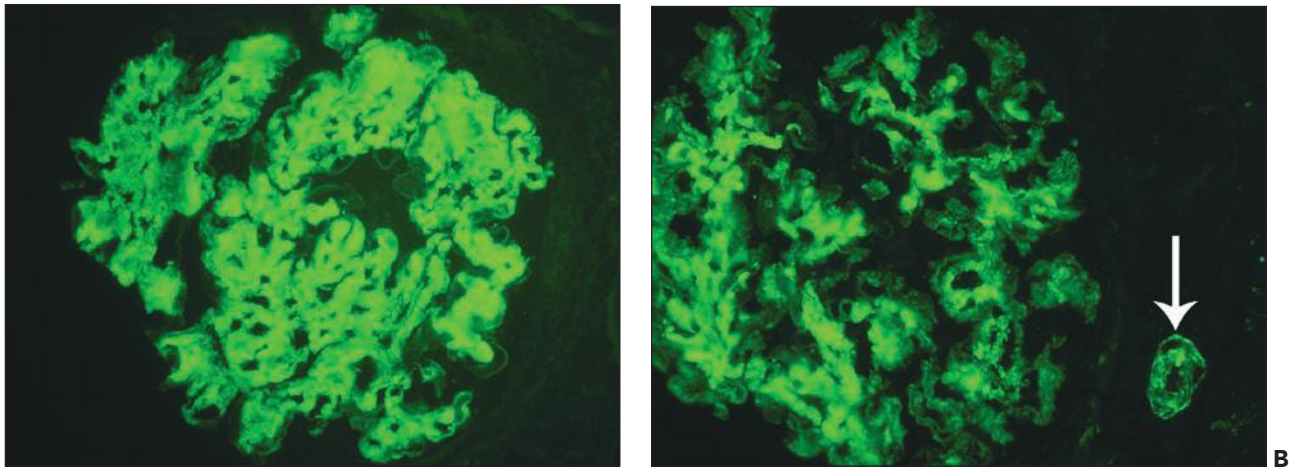


FIGURE 23.5 Fibrillary glomerulonephritis. A, B: Glomeruli illustrate the characteristic strong smudgy mesangial and capillary wall staining characteristic of FGN. **B:** Rare smudgy staining of the hilar arteriole (*arrow*). (Direct immunofluorescence, fluorescein isothiocyanate–labeled rabbit anti-human IgG, 400×.)

deviation of 7.4 nm (12) (Fig. 23.6). The fibrils are often set in a background of amorphous immune complex–type electron-dense deposits. Occasionally, the fibrillary deposits infiltrate the glomerular basement membrane and produce perpendicular spicular projections similar to those seen in amyloidosis. In rare cases corresponding to a membranous pattern by light microscopy, subepithelial deposits are present with little or no infiltration of the glomerular basement membrane seen in most cases (18,19). Tubular basement membrane deposits are very rarely present (18–20,31).

Differential Diagnosis

Although theoretically all entities included in this chapter enter in the differential diagnosis of FGN, the only serious consideration at the ultrastructural level is amyloidosis. As indicated above, amyloidosis is discussed in detail in a separate chapter;

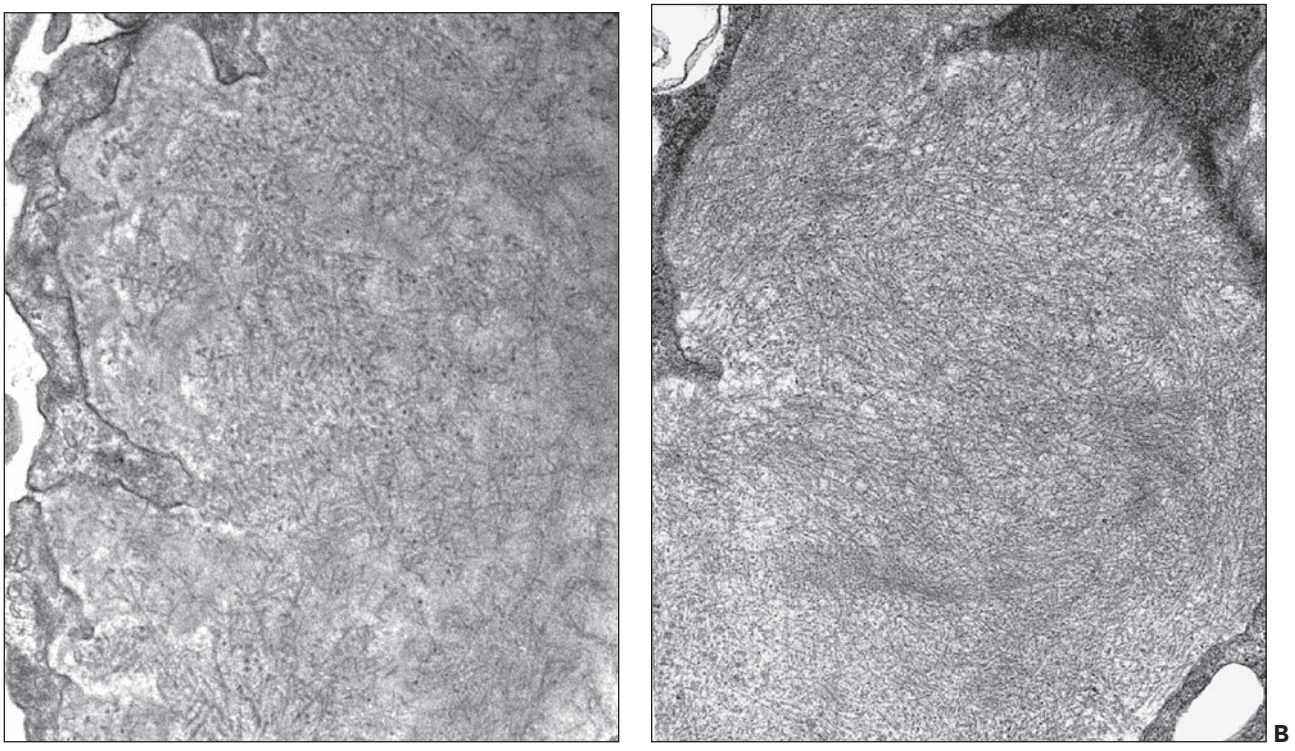


FIGURE 23.6 Fibrillary glomerulonephritis and amyloidosis. A: Electron micrograph of FGN deposit illustrating the randomly disposed nonbranching fibrils resembling amyloid fibrils but noticeably thicker. **B:** Electron micrograph of amyloidosis to contrast the thickness of the fibrils with those of fibrillary glomerulonephritis. (Uranyl acetate and lead citrate, 25,000×.)

however, this is an appropriate place to mention that of the long list of amyloidogenic proteins provided in Table 23.1, the ones likely by far to involve the kidney are AL amyloidosis and AA amyloidosis although recently cases of leukocyte chemotactic factor 2 amyloid have been reported with increasing frequency (32) in particular in patients of Latino heritage. Leukocyte chemotactic factor 2 amyloid has a striking tendency to involve the interstitial compartment although the glomeruli and extraglomerular blood vessels are not spared. Most other forms of amyloidosis rarely involve the kidneys.

Both amyloidosis and FGN do not pick up the silver component in the PAMS or Jones silver stain, and according to one group (18), the H&E stain and the PAS stain show weakly eosinophilic and equally weakly PAS-reactive mesangial material. This would make FGN indistinguishable from amyloidosis by light microscopy using routine stains. However, this has not been our experience or that of other investigators who have found the mesangium in FGN to be eosinophilic and PAS reactive (19). The immunofluorescence findings of amyloidosis depend on the type of amyloidogenic protein involved; although even in cases of light chain–related amyloidosis, the immunofluorescence findings are not always reliable and can even be misleading if not corroborated by additional laboratory results. Although it is reasonable to resort to immunohistochemistry to determine the type of amyloidosis, when in doubt, a reliable tool is mass spectrometry performed on laser-dissected tissue (33).

At the ultrastructural level, both FGN and amyloidosis are characterized by randomly disposed fibrils, which in the case of amyloidosis range from 7 to 15 nm in thickness, whereas in the case of FGN, the thickness of the fibrils is roughly double that ranges at 15 to 30 nm (Fig. 23.6), making the results of histochemical stains for amyloidosis such as Congo red, thioflavin T, and immunofluorescence microscopy for immunoglobulin and complement critical in distinguishing between the two entities when the fibrils' thickness in a particular case is at the borderline between that of amyloidosis and FGN. Therefore, even if the block has been cut through and would not allow for additional sections to be obtained to be stained with Congo red, one of the sections already stained with H&E or PAS ought to be destained and restained with Congo red to ascertain the diagnosis, since one defining feature of FGN is its Congo red negativity despite the ultrastructural resemblance to amyloidosis.

Another pitfall in the diagnosis of FGN is encountered when, often in a background of focal segmental glomerulosclerosis or diabetic glomerulosclerosis, the fibrillary nature of the mesangial matrix becomes particularly prominent for unclear reasons (34–36), needless to say, the Congo red stain if obtained is negative. Distinction from FGN usually relies on the negative immunofluorescence studies. At the ultrastructural level, the fibrils in most cases tend to be curvilinear and run together in serpentine bundles that intersect, although we have seen cases where the fibrils appeared to be disposed randomly, adding to the diagnostic confusion. Awareness of the existence of such cases and correlation with the underlying light microscopic features and immunofluorescence findings should assist in resolving problems that they may pose. This is discussed in further detail and illustrated in a separate section below on altered mesangial matrix.

The most important differential diagnostic consideration is the entity to be discussed next, namely, immunotactoid glomerulopathy (ITG). Not as much because of the difficulty of distinguishing between the two entities but rather because of the extensive debate that has been going on for over a decade between those who believe that there is no need to distinguish between these two entities merely on morphologic grounds and the majority of renal pathologists and nephrologists who agree that these are two entities that are distinguishable on both morphologic and clinical grounds.

Pathogenesis

The pathogenesis of FGN is not well understood. However, based on the study of Iskandar et al. where they found that of 13 cases stained for subclasses of IgG all stained strongly for IgG4 and only 15% of cases in addition exhibited weak staining for IgG1, it appears that even when the IgG component of deposits in FGN is polyclonal, they demonstrate a degree of homogeneity that may be one of the factors that along with the concentrating microenvironment provided by the filtration function in the glomerulus contribute to the complexes forming fibrillary structures. It has been pointed out that a similar profile is seen in membranous glomerulopathy, which is not associated with formation of fibrillary structures. However, the dominance of IgG4 in membranous glomerulopathy is not as pronounced as in FGN. Although IgG4 is incapable of activating the complement cascade via the classical pathway, aggregates of IgG4 and IgG4-containing immune complexes can activate the alternate pathway of complement. A study using immunogold labeling demonstrated that the immunogold-labeled antibodies to IgG, C3, and amyloid P component, but not those similarly labeled antibodies to fibronectin, fibrillin, or collagen IV, localized along the fibrils of FGN, suggesting that amyloid P component may play a role in fibrillogenesis but not any of the other proteins (37).

Treatment and Prognosis

The treatment of FGN has so far consisted into administration of steroids with or without cytotoxic drugs, which has not been effective with the disease progressing within 2 to 4 years from diagnosis to ESRD in approximately 50% of cases. The only study that has tried to correlate histologic pattern with prognosis found a negative correlation between the presence of crescents and tubulointerstitial damage and outcome (18). There was also a correlation between the different patterns observed in that study and the risk of progression as well as the rate of progression to ESRD. Transplantation is a valid option since although the fibrils recur in ≥50% of grafts, the influence on the outcome of the grafts is minimal (38,39). Rituximab showed encouraging effects on proteinuria in three patients (40).

IMMUNOTACTOID GLOMERULOPATHY

Clinical Presentation and Epidemiology

The origin of the term immunotactoid glomerulopathy goes back to 1980, when Schwartz and Lewis (5) described the case of a middle-aged man presenting with the nephrotic syndrome whose biopsy revealed mesangial and capillary wall deposits of IgG3-κ and ultrastructural examination showed mesangial and subepithelial microtubular deposits with a diameter of approximately 27 nm. The authors therefore speculated that the organized microtubules were the result of crystallization of

the immunoglobulins in a manner analogous to tactoid formation in the case of sickle cell disease.

Immunotactoid glomerulopathy is 10-fold rarer than FGN such that in most large referral biopsy centers, its frequency amounts to about 0.1% of native biopsies accessioned. Virtually all cases described have been in Caucasians, the gender distribution is equal, and the incidence is highest in the elderly with most cases reported in patients greater than 50 years of age. In one of the largest series (20), the age of incidence was significantly more advanced (62 years ± 2) compared to that of cases of FGN (50 years ± 2). Its clinical presentation is similar to that of FGN with one very important feature that, in addition to the ultrastructural appearance, justifies in the view of most pathologists and nephrologists the distinction between the two entities, which is its high association with a serum or urine paraprotein and/or an overt lymphoproliferative disorder, frequently a chronic lymphocytic leukemia (CLL) (17,18,20). As an example, 6 of the 14 cases in the series of Bridoux et al. (17) had CLL, 1 had a small lymphocytic B-cell lymphoma, and 3 had a monoclonal gammopathy of uncertain significance (MGUS).

Pathologic Findings

Gross Findings

As in the case of FGN, there is no experience with autopsy examination of cases that have not been modified by dialysis or transplantation.

Light Microscopy

Most series describe cases with membranoproliferative-like features (Fig. 23.7) or features of atypical membranous glomerulopathy (17,18,20). Depending on the abundance of the

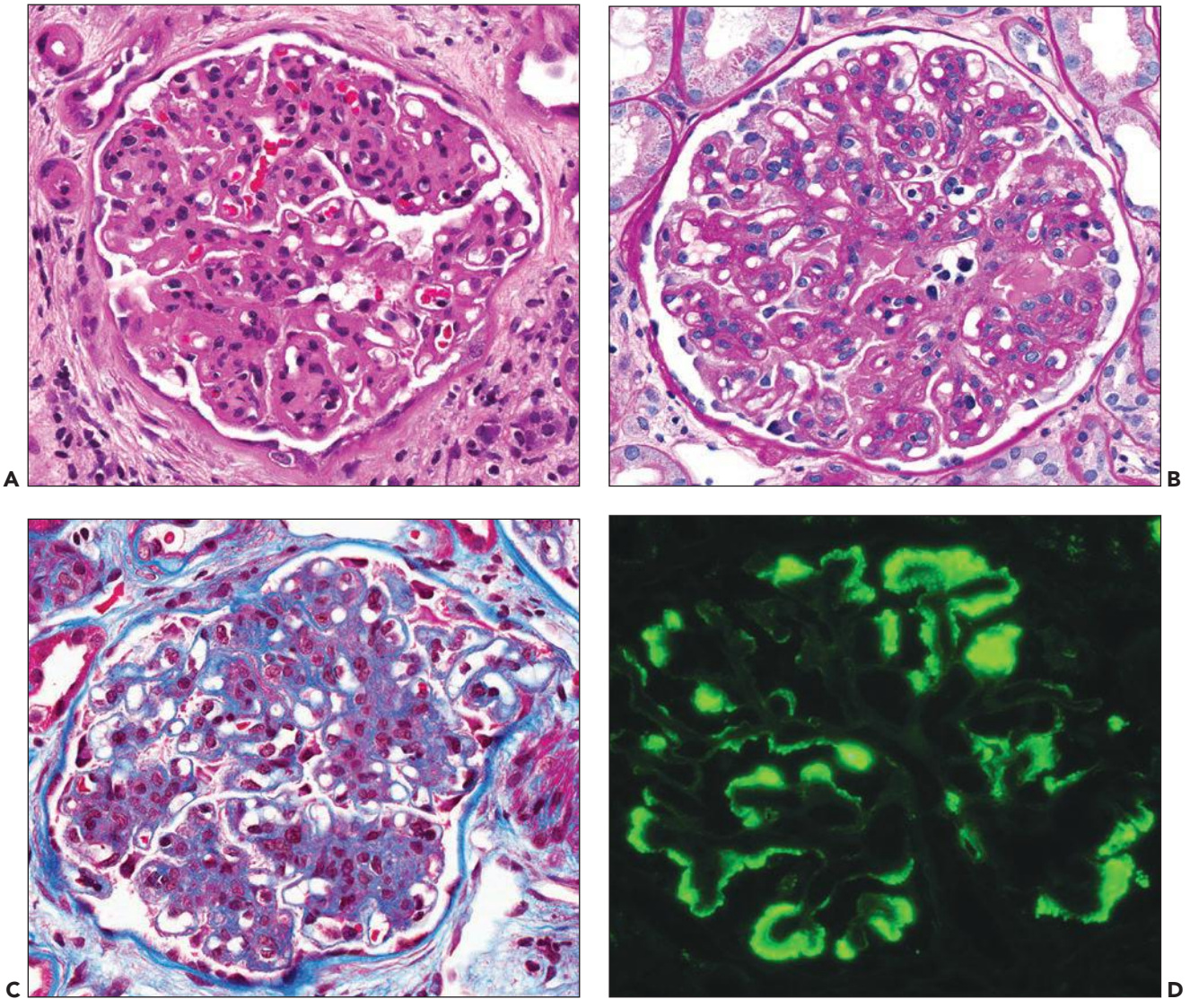


FIGURE 23.7 Monoclonal IgG lambda immunotactoid glomerulopathy with a membranoproliferative pattern with mesangial matrix expansion and hypercellularity, thick capillary walls, and slight lobulation **A:** H&E stain; **B:** PAS stain; **C:** Masson trichrome stain; **D:** Immunofluorescence microscopy showing capillary wall and mesangial staining for lambda light chains. Staining for IgG was similar, and staining for kappa light chains was negative. (400×.) (Courtesy of Carlos Andres Jimenez Guerrero and Adil Mohamed Hussein Gasim, Chapel Hill, NC.)

infiltrating microtubular material, the glomeruli may show focal segmental hyalinosis, and sometimes, intraluminal hyaline thrombi are present, representing aggregates of microtubules. None of the 6 cases reported by Fogo et al. (20) and Rosenstock et al. (18) demonstrated crescents, 2 of the 14 cases reported by Bridoux et al. (17) had crescents, and none of the 16 cases reported by the Mayo Clinic group had crescents (41). The trichrome-stained sections present an appearance similar to that described in connection with FGN. The sections stained with Jones stain are described as showing a moth-eaten appearance because of failure of the mesangium to pick up the silver stain. Of course, the stains for amyloid are negative.

Immunofluorescence Microscopy

All studies have found mesangial and capillary wall deposition of IgG and C3 with or without C1q. Monoclonality was present in 13 of 14 cases reported by Bridoux et al. (17) even though patients with known connective tissue diseases, light chain deposition disease, or cryoglobulinemic glomerulonephritis were excluded. This latter study was a multicenter study from France, which has a national health service, and therefore, when the investigators state that cases of cryoglobulinemic glomerulonephritis were excluded, it actually means that the patients were at the very least tested for cryoglobulinemia disregarding the problems with actually identifying cryoglobulins. This is different from the case in the study of Rosenstock et al. (18), which is based on a retrospective analysis of a database of a renal biopsy referral service where only 1 of 27 patients tested had a positive test. Yet, this still allowed some to find fault with the study as having claimed to have excluded patients with cryoglobulinemia, but "despite that claim, 56% of patients were not even serologically evaluated for the presence of cryoglobulinemia" (42). In the majority of cases, the monoclonal immunoglobulin is IgG-κ. In 10 of 14 cases in the series of Bridoux et al. (17), a subclass was determined revealing IgG1 in five cases, IgG2 in 3 cases, and IgG3 in 2. No cases stained for IgG4. Whereas in the same study, the IgG subclass was determined in three cases of FGN, showing two cases staining for IgG4 and one case staining for both IgG1 and IgG4.

Electron Microscopy

The main morphologic distinguishing feature is at the ultrastructural level where at the magnifications used in routine examination of renal biopsies instead of the randomly disposed straight fibrils seen in FGN, one clearly sees usually curved microtubules arranged in intersecting stacks in the mesangium and subendothelial and subepithelial compartment, depending on the histologic substrate of the particular case (Fig. 23.8). The individual microtubules can range from 10 to 90 nm although in any one case, the range is reported to be 25 to 35 nm. It appears that the lower spectrum of the range is seen in cases associated with CLL (17). There are two points to be emphasized in the above description: (a) the microtubular appearance is readily evident at the high magnification ranges used routinely in examination of renal biopsies (i.e., approximately 15,000× to 25,000×)—we stress this because some have argued that at extremely high magnifications, the fibrils of FGN can be shown to have electron-lucent cores—and (b) the arrangement of the microtubules in intersecting bundles/stacks, in contrast to the random disposition of fibrils forming a meshwork similar to that of amyloid although with thicker fibrils as is the case in FGN.

Differential Diagnosis

The cases that most resemble immunotactoid glomerulopathy are those of cryoglobulinemic glomerulopathy that have organized microtubules in the cryoglobulin deposits (Figs. 23.9 and 23.10), although not all cases of cryoglobulinemic glomerulonephritis have discernible microtubules. If the case meets the clinical and serologic criteria of cryoglobulinemia, the diagnosis is established. In most cases of cryoglobulinemia, the substructure is ill defined. However, in those cases where a microtubular substructure can be clearly discerned, the microtubules tend to be short and curved and are occasionally arranged in pairs.

Occasionally, cases of lupus nephritis may present difficulty if one looks at the ultrastructure in isolation. The deposits may have a microtubular appearance resembling that of cryoglobulins although the curves of the microtubules are more pronounced, leading to the term fingerprint appearance. The light microscopy can offer a membranoproliferative-like appearance with wire-loop lesions and hyaline thrombi—all features that can be shared with ITG. It is only the clinical

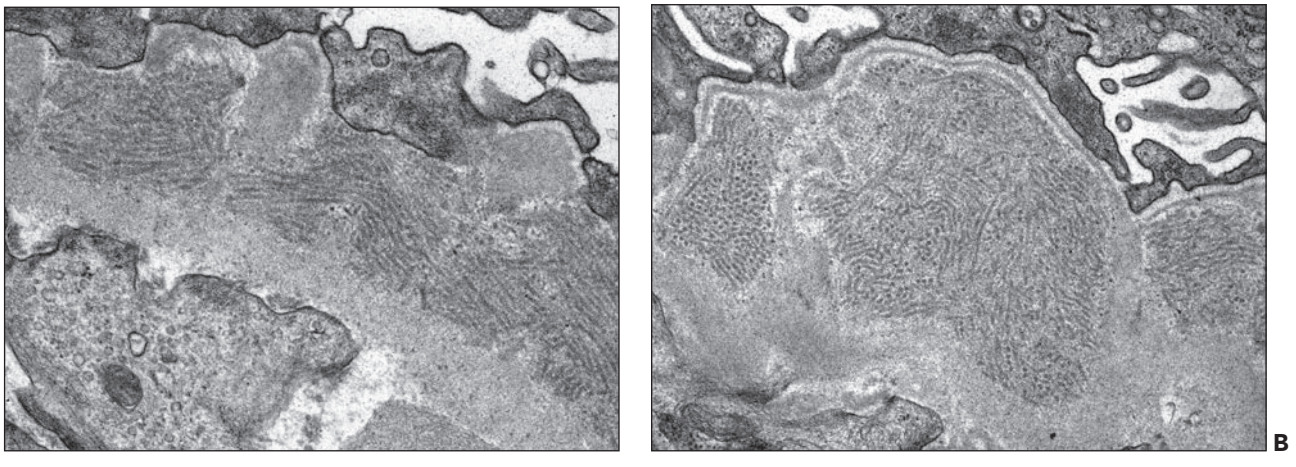


FIGURE 23.8 Monoclonal IgG lambda immunotactoid glomerulopathy (same case as in Fig. 23.7). Electron micrographs showing subepithelial parallel bundles of microtubules. Compare this pattern to the randomly arranged fibrils in Figure 13.6. (**A:** 15,000×; **B:** 20,000×, uranyl acetate and lead citrate.)

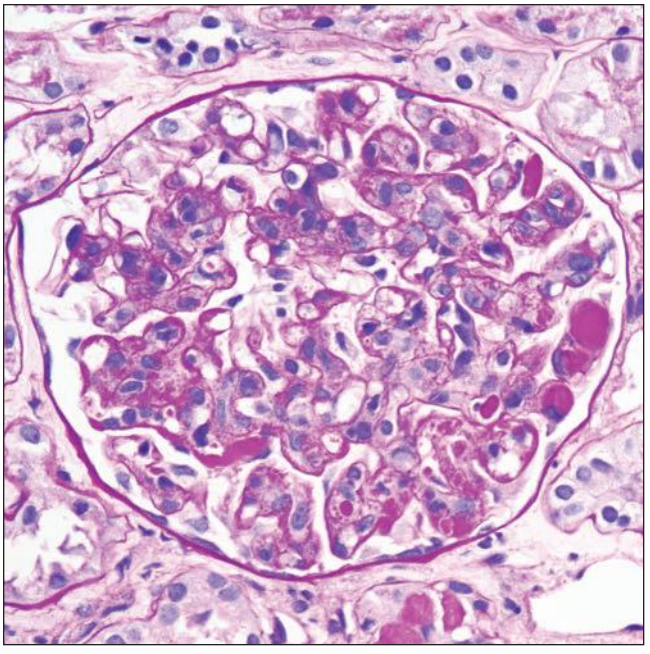


FIGURE 23.9 Cryoglobulinemic glomerulonephritis. PAS-stained glomerulus demonstrating segmental capillary wall thickening by PAS-positive deposits and several "hyaline thrombi" in capillary lumens (e.g., at 3:00). (500×.)

diagnosis of systemic lupus erythematosus that provides the unequivocal clue to the correct diagnosis.

FGN has been discussed in detail above; the only issue to be mentioned here regards the polemic that has gone back and forth since the emergence of ITG concerning whether both FGN and ITG should be considered one entity designated as immunotactoid glomerulopathy (FGN) or whether they should be viewed as separate entities (15–18,43,44). The preponderance of the evidence provided by investigators cited above supports the position that there is enough reason based on pathologic as well as clinical data to consider these two separate entities (Table 23.3) (17,18,20,45). Schwartz et al. (13) have emphasized that the diagnosis of ITG should be rendered only when all evidence of systemic diseases including collagen-vascular diseases, lymphoproliferative conditions, and cryoglobulinemia have been excluded; yet, their own series included two patients with high antinuclear antibody titers, one of which was considered to have mixed connective tissue disease with symptoms most consistent with scleroderma, and a third patient had CLL. Further, although no evidence of a paraprotein may be present at the time of biopsy, it may emerge during follow-up (46). The same can be said regarding cryoglobulinemia (18). Further clinical differences between the two entities include that the age of onset of ITG is, on average, a decade later than in is the case for FGN and that the prognosis is not as poor in ITG as it is in FGN. In conclusion, based on the above evidence, the distinction between FGN and ITG is both pathologically justified and clinically relevant.

Pathogenesis

The pathogenesis of ITG is not fully elucidated although, since in the majority of cases the immunoglobulins involved are monoclonal; this suggests that the combination of the homogeneity of the immunoglobulins involved with the added concentrating effect of filtration in the glomerulus plays a role in fibrillogenesis. Nasr et al. group used the technique of laser microdissection liquid chromatography and tandem mass spectrometry on three cases and detected, in addition to the immunoglobulins and monotypic light chains expected, evidence of a classic pathway of complement activation including C4 and C3 and the terminal pathway components; surprisingly, however, a number of

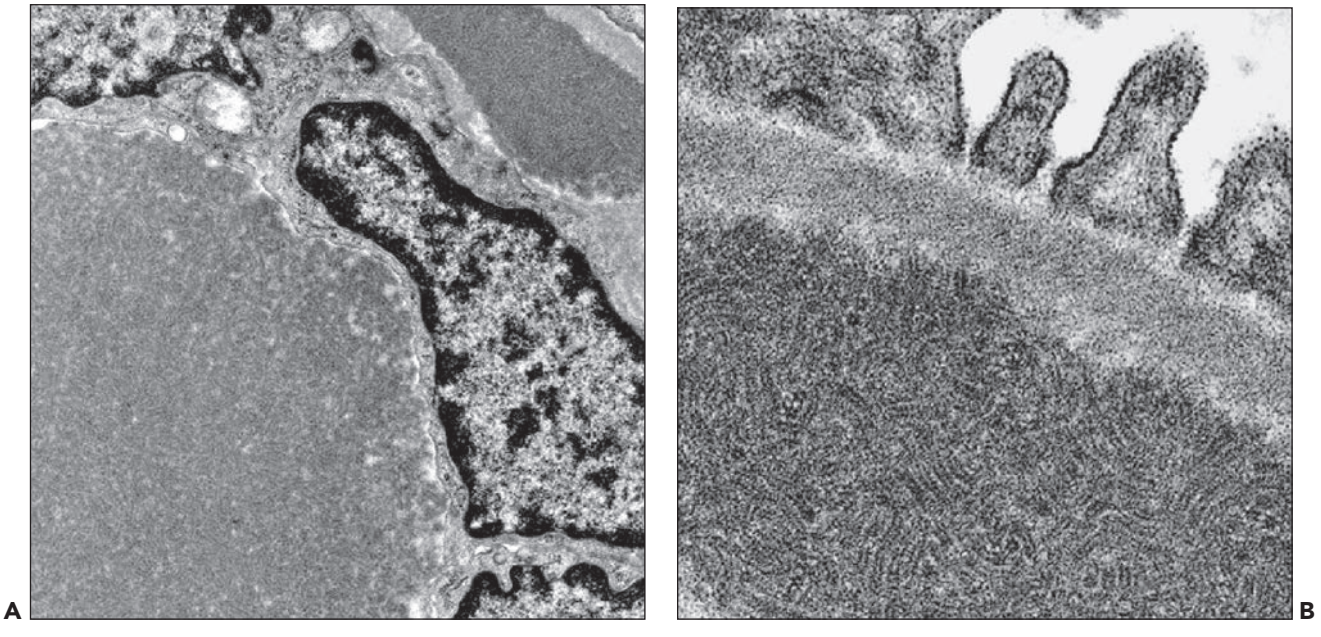


FIGURE 23.10 Cryoglobulinemic glomerulonephritis. A: At the **lower left** is an intraluminal coagulum of cryoglobulins ("hyaline thrombus") with an adjacent endothelial cell with underlying subendothelial cryoglobulin deposit. (10,000×.) **B:** Higher magnification of the subendothelial deposit in (**A**). Note the short *curved* microtubules seen as annular-shaped structures were sectioned transversely. (30,000×; uranyl acetate/lead citrate staining.)

TABLE 23.3 Distinctions between fibrillary glomerulonephritis and immunotactoid glomerulopathy

	Fibrillary glomerulonephritis	Immunotactoid glomerulopathy
Immunofluorescence microscopy	Deposits of polyclonal IgG4 and less often IgG1 with staining for both κ and λ light chains and only a small minority of cases monoclonal, typically staining for IgG-κ	Majority of cases have monoclonal deposits staining for IgG1, IgG2, or IgG3 and either κ or λ light chains
Electron microscopy	Randomly disposed fibrils that form a meshwork resembling that of amyloidosis but distinguishable by the greater thickness of the fibrils	Microtubules are readily seen to have electron-lucent cores without the need for extremely high magnifications and are most often arranged in parallel bundles/stacks that course in intersecting planes
Clinical features	Rare association with lymphoproliferative malignancies or plasma/urine monoclonal immunoglobulins	Frequent association with lymphoproliferative malignancies or plasma/urine monoclonal immunoglobulins

amyloid-associated serum proteins were found including serum amyloid P component, apolipoprotein E, and clusterin (41).

Treatment and Prognosis

In one series, ITG was treated with corticosteroids alone, or associated with cyclophosphamide or melphalan, or with cyclophosphamide and vincristine or doxorubicin, or with chlorambucil, or chlorambucil alone (17). In 10 of 12 nephrotic patients, partial or complete remission was achieved. The conclusion of the authors was that aggressive treatment may lead to remission of the nephrotic syndrome in many patients, which may have a favorable influence on the long-term renal prognosis. Although we should add that this improved quality of life is not necessarily accompanied by an equally favorable effect on patient survival since compared to the group with FGN in the same study after a follow-up period of 54 months for ITG and 56 months for FGN, patient survival was similar for the two groups (71.4% vs. 88.8%, respectively, $P = 0.62$).

CRYOGLOBULINEMIC GLOMERULONEPHRITIS

This topic is dealt with in detail in Chapter 22; it is discussed here in a summarized form for differential diagnostic purposes. Cryoglobulins are immunoglobulins that are soluble at 37°C and precipitate at 4°C. In 1966, Meltzer et al. (47) identified the presence of rheumatoid factor activity in cryoglobulins and mentioned the presence of proliferative glomerulonephritis in some of the patients. In 1974, Brouet et al. (48) proposed the classification currently used for cryoglobulins. According to that classification, type I (25%) cryoglobulins are monoclonal proteins that have no antibody activity and are usually associated with a lymphoma or with multiple myeloma, although some are associated with MGUS. They precipitate when their concentration increases and are therefore associated with the hyperviscosity syndrome. They rarely involve the kidney, although as many as 18 cases have been reported associated with a membranoproliferative-like glomerulonephritis, the pattern typically seen in cryoglobulinemic glomerulonephritis, and in two cases, the isotype of the monoclonal immunoglobulin was reported to be IgG3. Type II cryoglobulins (25%) are mixed and are composed of a monoclonal IgM with a rheumatoid activity directed to the Fc part of polyclonal IgG. A high proportion of these cases are associated with chronic hepatitis C infection, and other cases are associated with connective tissue diseases. Type III cryoglobulins (50%) consist of polyclonal or oligoclonal IgM with rheumatoid factor activity to polyclonal IgG. About 30% of cases are associated with chronic hepatitis C infection, while the rest are associated with chronic infections such as chronic hepatitis B antigenemia and with connective tissue diseases. Only a small fraction of types II and III are currently of unexplained etiology and deserve the term mixed essential cryoglobulinemia. The majority of cases of cryoglobulinemic glomerulopathy are associated with type II cryoglobulinemia (49).

Pathologic Findings

Light Microscopy

The usual features are those of a membranoproliferative glomerulonephritis type I with diffuse global endocapillary hypercellularity, associated with accentuation of the lobular architecture. The cells contributing to the increased cellularity can be shown by immunohistochemistry to be predominantly macrophages reacting with CD68 in addition to mesangial cells. The PAMS stain often shows peripheral mesangial interposition with a tram-track pattern. The H&E and PAS-stained sections disclose segmental to global thickening of the capillary walls. The diagnostic feature is the identification of the so-called hyaline thrombi (Fig. 23.9), which can be shown by ultrastructural examination and immunofluorescence microscopy to consist of aggregates of cryoglobulins.

Immunofluorescence Microscopy

The immunofluorescence patterns are highly variable. There can be granular deposits along the capillary walls, strong staining of the subendothelial deposits and the hyaline thrombi, or only staining of the periphery of the hyaline thrombi, if present. Staining reflects the composition of the circulating cryoglobulins, typically IgG and IgM, and sometimes dominance of κ light chains (50,51).

Electron Microscopy

As already discussed above in the differential diagnosis of ITG, the aggregates of cryoglobulins can be amorphous or can demonstrate an ill-defined microtubular substructure or in exceptional cases can demonstrate well-defined short curved microtubular (cylindrical) structures with electron-lucent cores and circular (annular) profiles that represent cross-sections of the microtubules (Fig. 23.10). The ultrastructural appearance of cryoglobulins precipitated from the circulation corresponds to that of cryoglobulin aggregates in glomeruli.

FIBRONECTIN GLOMERULOPATHY

Clinical Presentation and Epidemiology

This entity was first reported by Burgin in 1980 in three siblings and a first-degree cousin (7). A few other families have since been reported with this rare condition. The pattern of inheritance is autosomal dominant in the majorities of the families (52). Patients have presented clinically at different ages, although most are diagnosed between the ages of 15 and 30 years. The clinical presentation is characterized by proteinuria, often nephrotic range, microscopic hematuria, renal tubular acidosis type IV, and hypertension. At the time of presentation, less than half of these patients exhibit some degree of renal insufficiency. No systemic manifestations have been reported. Serum fibronectin levels are typically normal.

Pathologic Findings

Light Microscopy

The characteristic finding is enlargement of glomeruli with markedly increased mesangial extracellular PAS-positive material, variable capillary wall thickening, and a lobular pattern (Fig. 23.11A). The expanded mesangial areas and thick capillary walls are typically intensely red with the trichrome stain (Fig. 23.11B). However, the expanded mesangium does not stain with the silver in the periodic methenamine silver stain/Jones stain and is not congophilic. Glomerular hypercellularity, peripheral mesangial interposition, necrosis, and crescents are absent. Tubulointerstitial damage and vascular changes only supervene as glomerular lesions progress to global glomerular sclerosis.

Immunofluorescence Microscopy

The conventional immunofluorescence staining panel shows no significant deposits except in a minority of cases that have mesangial staining for IgG, IgM, and C3. On the other hand, immunohistochemical staining for fibronectin reveals mesangial staining in all cases tested.

Electron Microscopy

The ultrastructural findings are characterized by the presence of large subendothelial and mesangial electron-dense deposits (Fig. 23.12). Extraglomerular deposits have been described in a few cases along the Bowman capsule and tubular basement membranes. The electron-dense deposits are generally composed of distinct electron-dense material that is either amorphous or granular (most cases) or vaguely fibrillary, and in selected cases, both types are noted intermingled. Fibrillary material is often seen only focally in a background of amorphous to granular electron-dense material. The fibrils are generally short, often arranged in a compact fashion, and measure between 10 and 14 nm in diameter. The amount of electron-dense material deposited in the mesangium is quite variable from case to case; however, it can be massive. This material replaces the normal mesangium.

Differential Diagnosis

Because the main finding is mesangial expansion and associated accentuation of lobularity, there are many diseases that can mimic fibronectin glomerulopathy at the light microscopic level. Since this is a rare disease, it is typically not suspected unless the family history of a confirmed case is provided. The immunofluorescence findings using the routine panel of stains are nonspecific and do not provide a specific indication of the diagnosis but rule out diseases that have prominent immunoglobulin and/or complement deposits. The main diagnostic features that suggest this diagnosis are at the electron microscopic level and are suggestive though not diagnostic. The immunohistochemical stain for fibronectin demonstrating intense staining of the deposits confirms the diagnosis and confirms the ultrastructural suspicion.

Pathogenesis

The kidney produces fibronectin that accumulates in the mesangium, and the liver generates circulating fibronectin. Production of fibronectin occurs in the mesangium by mesangial cells, and its production is a common finding accounting for expansion of the extracellular matrix in experimental platforms. Up-regulation of fibronectin production has been documented to occur in several conditions in humans (53). Plasma levels of fibronectin are not elevated in patients with fibronectin glomerulopathy (54); therefore, this disease appears to result from renal dysfunction. There is either a problem with clearing of fibronectin, perhaps due to the formation of a variant of fibronectin that cannot be cleared, or, alternatively, a circulating factor becomes attached to circulating fibronectin that reaches the renal mesangium and cannot be cleared (55). This has been observed to occur in the uteroglobin knockout mouse model (56), though involvement of the uteroglobin gene has been ruled out in humans (57). One proposed mechanism is that there is a defect in the catabolism of fibronectin. In essence, the pathogenetic mechanisms involved in this disorder have not been conclusively elucidated. Vollmer et al. mapped the gene to 4.1-cm interval on chromosome 1q32, and they have proposed a candidate gene from the regulation of complement activation cluster that is localized to this region. Amyloid P has been localized to the fibrillary deposits in this condition in one case, suggesting a probable connection between amyloid P component and fibrillogenesis (58). Proteomic analysis using laser capture microdissected glomeruli from renal biopsies of patients with fibronectin glomerulopathy has demonstrated the accumulation of fibronectin and fibulin in the mesangium (59).

Treatment and Prognosis

There is no specific treatment for this condition. Supportive care can be provided to ameliorate proteinuria and control hypertension. Slowly progressive deterioration of renal function occurs in the majority of the cases. The patient may progress to end-stage disease as early as in the second and as late as in the sixth decade of life. Thirteen affected members of a kindred all progressed to ESRD (57), but this has not been the case in other kindreds where the progression to renal failure has been variable and impossible to predict.

For the most part, excellent results have been obtained with kidney transplantation in these patients, but in a few cases, fibronectin deposits have appeared in the transplanted kidney as early as 19 days after transplantation (59). In one case with proven recurrence, the allograft was eventually lost (60).

DIABETIC FIBRILLOSIS

Clinical Presentation and Epidemiology

Unusually prominent extracellular matrix collagen fibrils (fibrillosis) occur in a very small subset of patients with nodular diabetic glomerulosclerosis and can only be seen by electron microscopy. It was first reported by Sohar et al. (61) in 1970 who coined the

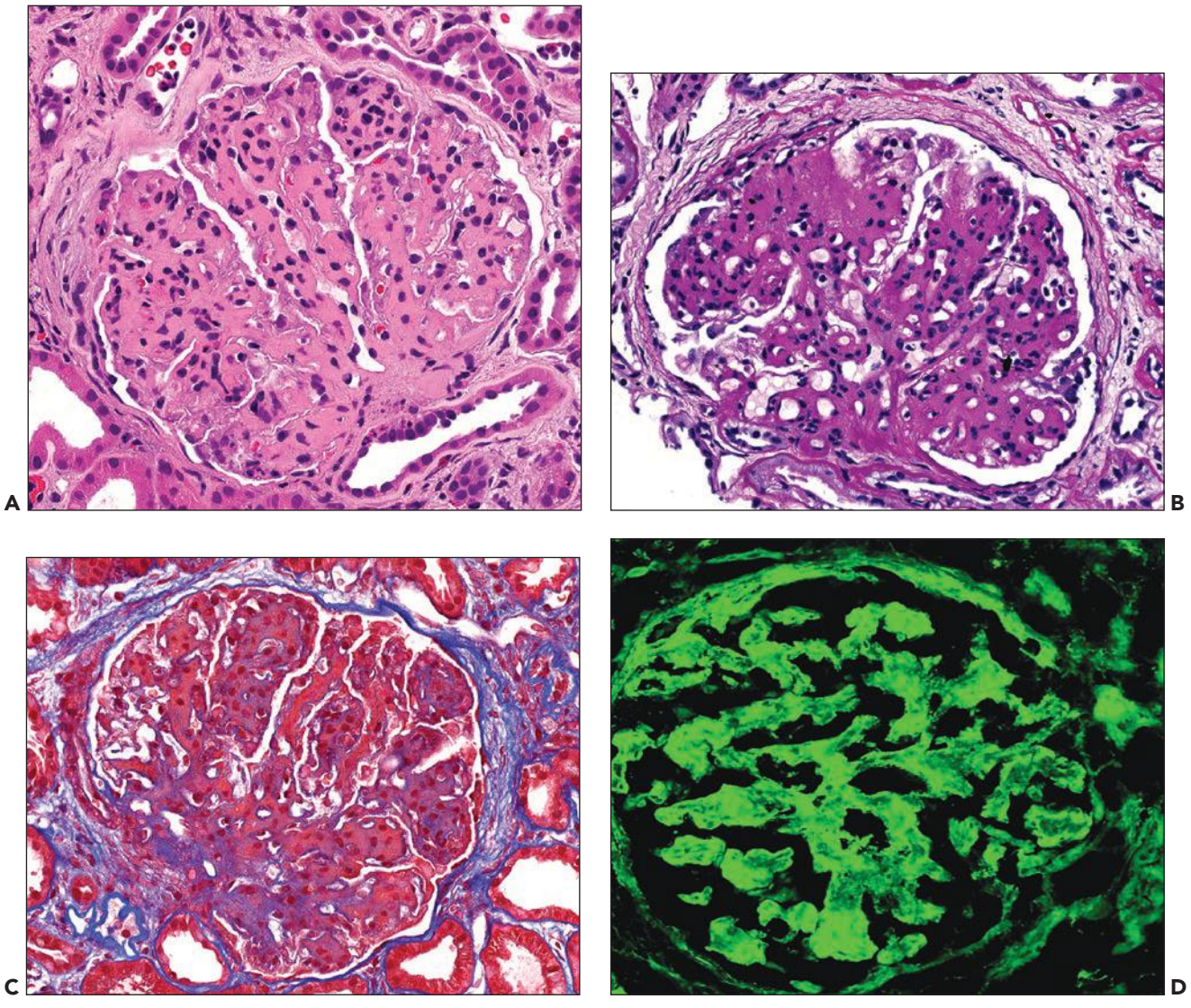


FIGURE 23.11 **Fibronectin glomerulopathy.** Mild hypercellularity with an expanded mesangium that is PAS positive and stains red with the trichrome stain (**A:** H&E stain; **B:** PAS stain; **C:** Masson trichrome stain; **D:** Immunofluorescence microscopy with antifibronectin. (500×.))

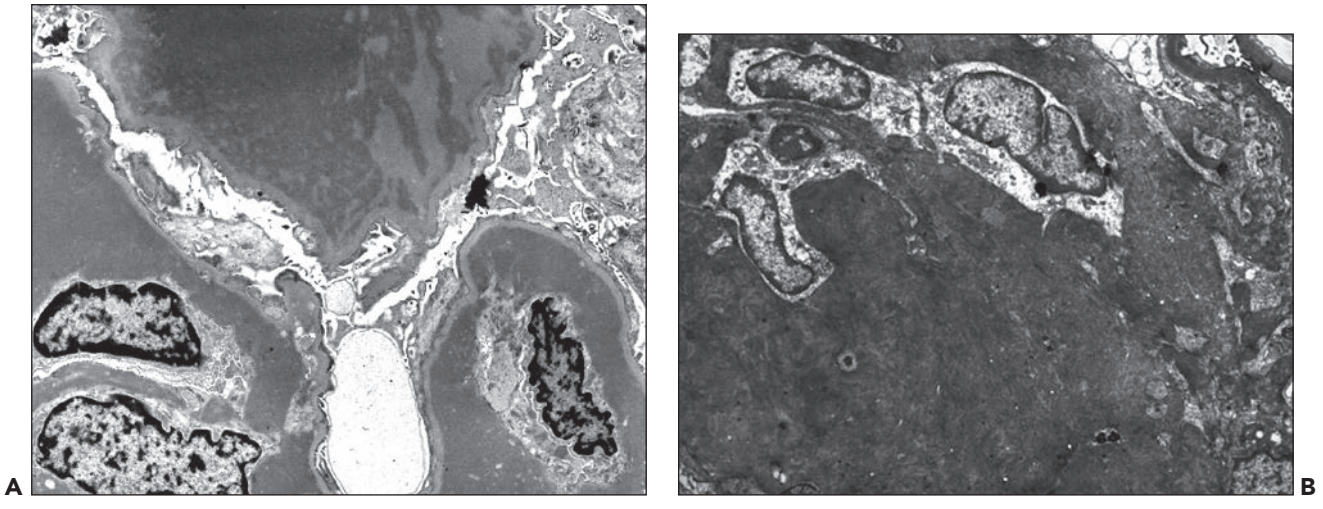


FIGURE 23.12 **Fibronectin glomerulopathy. A:** Electron-dense material expanding the mesangium. (10,000×). **B:** Higher magnification of a different case showing a vague texture of the deposits. (22,500×.)

term diabetic fibrillosis. They found fibrillary deposits in the glomeruli and vessel walls in virtually every organ examined in three autopsies from diabetic patients. Diabetes mellitus was diagnosed in these patients at 30, 40, and 44 years of age. They all had nephrotic range proteinuria (5 to 9 g/d) at the time of death and were hypertensive. When manifestations of this disorder became apparent in two of these patients, the diabetes mellitus was of relatively short duration and mild, and in the other patient, it preceded the diagnosis of diabetes mellitus. Fibrillosis appears to be a variant expression of the glomerular, vascular, and other sclerosis caused by diabetes and has no recognized distinct pathogenesis or association with a particular clinical presentation or course. The most important issue for pathologists is not to confuse this process with one of the specific variants of glomerular disease with organized deposits, for example, FGN or amyloidosis.

Pathologic Findings

Gross Pathology

In the original series, the kidneys were reported to be enlarged in two cases (no weight provided) and small (90 g each) in the other case (61). No other gross pathologic studies of this entity have been performed.

Light Microscopy

Deposits of homogeneous material have been described in vessels of all calibers and in the connective tissue of many internal organs and in the kidney in the first three cases. The deposition in the kidneys was along glomerular basement membranes and in mesangial matrix, as well as surrounding tubular basement membranes. This material stains bright with the H&E and PAS stains and positive with the colloidal iron stain (61,62). In some instances, it replaced entirely the walls of venules and arterioles and at times resulted in luminal narrowing. In the glomeruli, typical nodular glomerulosclerosis is seen, and focally, in some of the mesangial nodules, similar material can be detected, but often, it is buried in the expanded mesangium. Characteristically, the mesangial nodules maintain their silver positivity with scattered nonstaining spots sometimes detectable depending on how much fibrillary material is present (Fig. 23.13). Similar material has been documented in the heart, spleen, and liver. The deposits are Congo red (62) and Thioflavin T negative.

Electron Microscopy

Randomly deposited, nonbranching fibrils measuring approximately 10 to 25 nm in diameter are noted in a background of mesangial expansion with nodular accentuation with increased matrix and thickening of the glomerular basement membranes, classical findings in nodular diabetic glomerulosclerosis (Fig. 23.14).

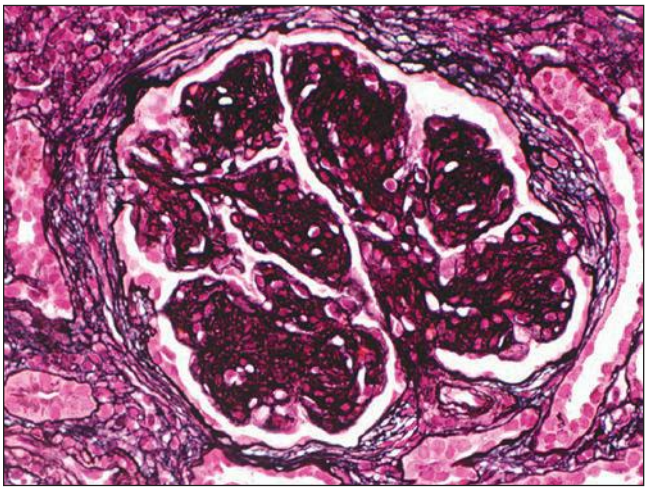


FIGURE 23.13 Diabetic fibrillosis. Expanded mesangium with abundant silver-positive material. Nonstaining spots are also evident in the expanded mesangium. (Jones silver methenamine stain, 500×.)

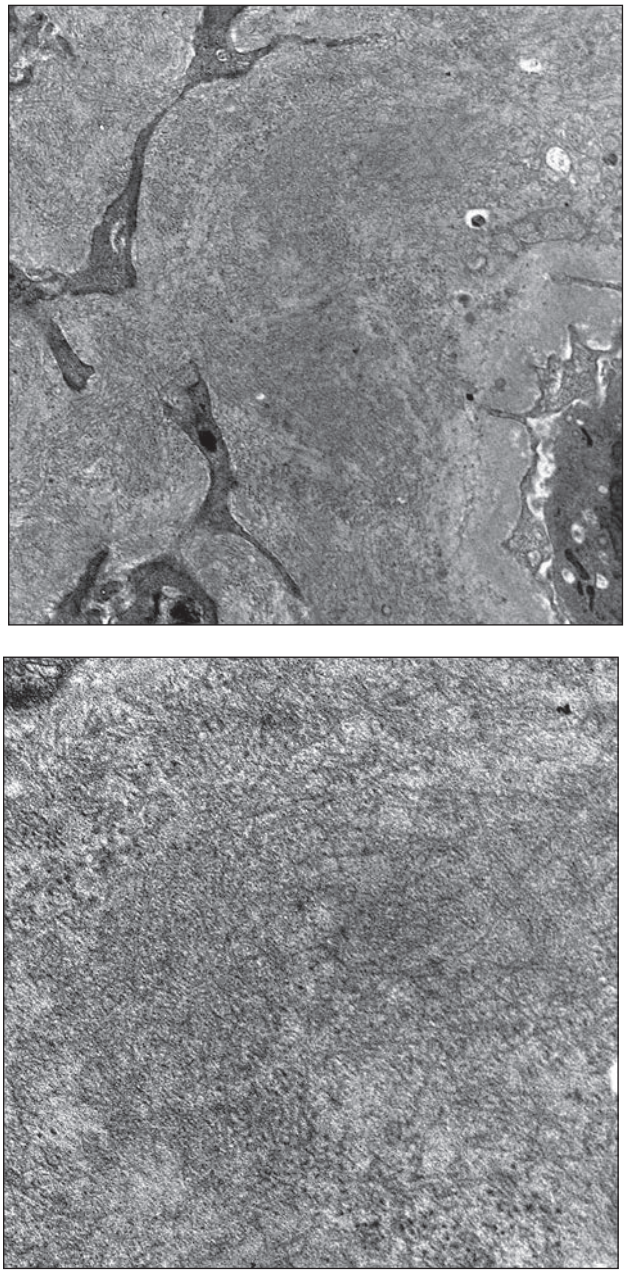


FIGURE 23.14 Diabetic fibrillosis. A: Expanded mesangium with increased matrix and randomly arranged, nonbranching fibrils. (20,500×.) **B:** Higher magnification. (51,300×) (TEM, uranyl acetate and lead citrate).

Differential Diagnosis

Differential diagnosis includes FGN and amyloidosis. In diabetic fibrillosis, the mesangial nodules maintain their silver positivity, while in the other two entities, the normal mesangium is replaced by fibrils, and therefore the mesangium loses its silver positivity and is characterized by alternating areas that are positive and others that are negative (a moth-eaten appearance). Typical immunofluorescence findings of FGN with smudgy deposits containing IgG, C3, kappa, and lambda light chains are lacking. There have been cases of coexistent diabetic nephropathy and FGN reported in the literature, and this combination of diseases must be ruled out (63,64). In the cases of amyloidosis, Congo red with apple green birefringence upon polarization of positive tissue and Thioflavin T positivity is present and absent in the other two conditions. Fibrils of diabetic fibrillosis are an extreme example of the accentuated collagen fibrils that occasionally occur in nondiabetic glomerular sclerosis (36). The accentuated collagen fibrils in fibrillosis differ from the abnormal glomerular collagen deposits of collagenofibrotic glomerulopathy by not having the periodicity (banding) that is seen in the predominant type III collagen of collagenofibrotic glomerulopathy.

Pathogenesis

Local physicochemical conditions in the mesangium and other sites of excess extracellular matrix production in diabetes likely contribute to the development of the accentuated fibrillary texture of the collagenous matrix.

Clinical Course and Prognosis

Patients with diabetic fibrillosis occurring in the setting of nodular diabetic glomerulosclerosis have the same clinical course as patients with typical nodular diabetic glomerulosclerosis.

COLLAGENOFIBROTIC GLOMERULOPATHY (COLLAGEN III GLOMERULOPATHY)

This topic is dealt with in a separate chapter that deals with the Alport syndrome, thin basement membrane nephropathy, and the nail-patella syndrome. It is mentioned here for comparison to other glomerular diseases with organized deposits.

Clinical Presentation and Epidemiology

This entity was first reported in 1979 by Arakawa et al. (65) at the annual meeting of the Japanese Society of Nephrology. They recognized a similarity with nail-patella syndrome but emphasized the absence of skeletal abnormalities as a differentiating feature (65). Dombros and Katz (6) published a case of this rare entity in 1982. Ikeda et al. first identified the peculiar collagen fibers identified in the glomeruli of these patients as collagen type III and termed the disease primary glomerular fibrosis. Arakawa et al. (66) documented 10 similar cases and coined the term collagenofibrotic glomerulopathy. In 1995, collagenofibrotic glomerulopathy was included in the World Health Organization classification of glomerular disorders, as a distinct type of glomerular disease. Since then, about 40 additional cases with this rare glomerular disorder have been published under different names including primary glomerular fibrosis, collagen III glomerulopathy, and collagenofibrotic glomerulopathy. The first three cases from Latin America were published in 2009 (67).

The reported patients have ranged in age from 6 to 72 years and have exhibited no sex predilection. Most patients have presented in the fourth to seventh decade of life. Many of the patients reported in Japan have been adults whereas those reported in Europe have been children, suggesting different genetic patterns of penetrance of the disease. The pattern of inheritance appears to be autosomal recessive, differing from the autosomal dominant pattern that is characteristic of the nail-patella syndrome. In one case, there was an association with factor H deficiency (68), and another case was associated with Hodgkin lymphoma (69). One case was associated with multiple exostosis and mutation of the exostosin-1 (EXT1) gene (70). Reviews of collagenofibrotic glomerulopathy clarify the characteristic pathologic and clinical features (71,72).

The initial symptoms generally occur early in infancy or late childhood, and the main presentation is proteinuria with edema. The proteinuria may be in the nephrotic range. Renal functions tests are often normal, and when that is not the case, the alterations are usually mild. Other clinical manifestations may include hypertension or hematuria, often microscopic (73). In at least one family, the presenting clinical finding was hematuria. No systemic manifestations appear to be present in the majority of these patients. One patient developed hepatic perisinusoidal fibrosis (74), and another one exhibited massive accumulation of collagen III in the kidney and in many organs including the spleen, liver, myocardium, and thyroid gland (75).

Pathologic Findings

Light Microscopy

The glomeruli appear enlarged and in some cases exhibit an appearance that resembles membranoproliferative glomerulonephritis, because of their accentuated lobularity, thick capillary walls, and partial obliteration of capillary lumens (Fig. 23.15A), though cellular proliferation is not a prominent feature in this condition. Rarely, small epithelial crescents may be seen. Double-contoured capillary walls are seen along some peripheral capillary walls. Mesangial areas are expanded and contain PAS-positive material that stains blue with the trichrome stain (Fig. 23.15B and C), representing increased mesangium, which is biochemically altered (6,8,65,66,71,72). In the advanced stages, mesangial nodules similar to those in diabetic nephropathy may be seen (76).

Using immunohistochemistry, collagen III is demonstrated in the prominent mesangial areas that react weakly with silver methenamine and in the subendothelial zones of some peripheral capillary walls (77–88). There is also immunoreactivity for collagen I in some cases, and in one case, strong costaining for collagen V was shown (89). A very small amount of collagen III or none at all is seen in normal glomeruli (90). Congo red and Thioflavin T stains are negative.

Immunofluorescence Microscopy

The findings are rather inconstant, but focal and segmental granular IgG and IgM deposition has been documented but felt to be a result of trapping, as well as interrupted staining for C1q along peripheral capillary walls, in at least some cases within areas with segmental hyalinosis. Other immunoreactants are characteristically negative, including kappa and lambda light chains.

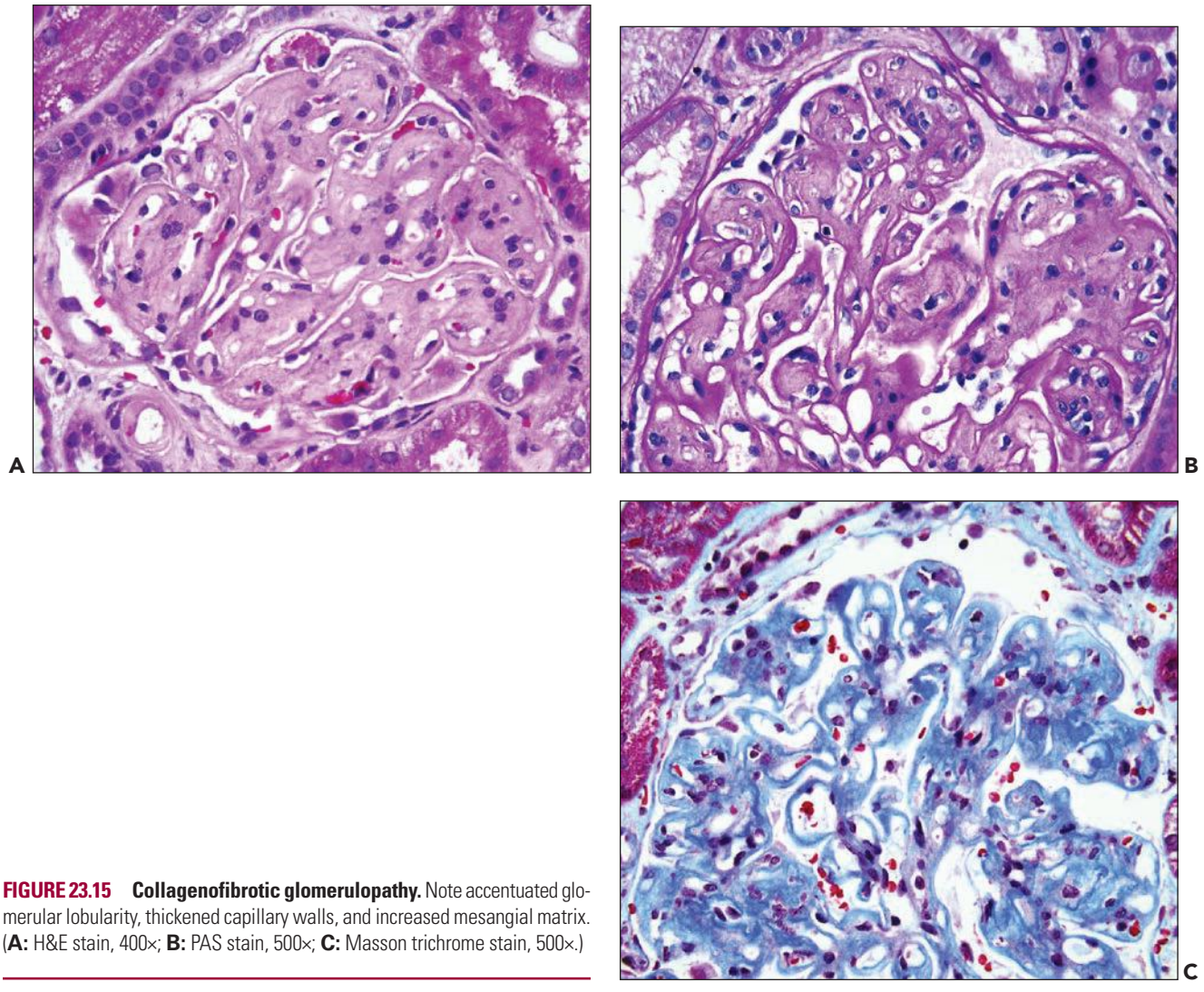


FIGURE 23.15 Collagenofibrotic glomerulopathy. Note accentuated glomerular lobularity, thickened capillary walls, and increased mesangial matrix. (**A:** H&E stain, 400×; **B:** PAS stain, 500×; **C:** Masson trichrome stain, 500×.)

Electron Microscopy

Ultrastructural evaluation is useful to establish a definitive diagnosis. Ultrastructural findings usually are the indication for staining for collagen III to confirm the diagnosis. Fibers with periodicity are identified in the expanded mesangial areas and along involved subendothelial zones. The fibers exhibit a distinctive electron microscopic appearance. They are commonly curved and frayed, and their periodicity (banding) is distinct. The fibers have a transverse band structure with a periodicity of 43 to 65 nm (72), as has been characteristically associated with collagen III. In some cases, the fibers appear worm-like or comma-like when sectioned transversely (91) and in others reveal a peculiar shape that is quite unique (9) (Fig. 23.16). This is in contrast to typical collagen fibers that are less curved and typically dispose themselves in an organized, parallel fashion. Fibers are not identified within the lamina densa of glomerular basement membranes, as is typically present in nail-patella syndrome.

Although the fibers are seen in routine ultrastructural preparations, they are much better visualized when stained with tannic acid lead or phosphotungstic acid (76). No immune complexes are classically present, but one case with immune complexes has been published (92); however, this probably was a result of coexistence with a second glomerular immune complex–mediated process.

Differential Diagnosis

Due to the light microscopic appearance, the differential diagnosis includes entities such as membranoproliferative glomerulonephritis, light chain deposition disease, diabetic nephropathy, FGN, chronic phase of thrombotic microangiopathy, and other lobular glomerulopathies. While the light microscopy and immunofluorescence microscopy profiles are helpful in differentiating some of these conditions, the ultrastructural findings should suffice to suggest or make a definitive diagnosis of collagen III glomerulopathy. In approximately one third of patients with other glomerular diseases, there may be focal and weak staining for collagen III quite unlikely the strong and distinct staining seen in collagenofibrotic glomerulopathy (76,93).

Pathogenesis

The pathogenesis of this disorder remains enigmatic. However, ethnic or genetic and possibly environmental factors appear to play an important role. Normal human glomeruli essentially

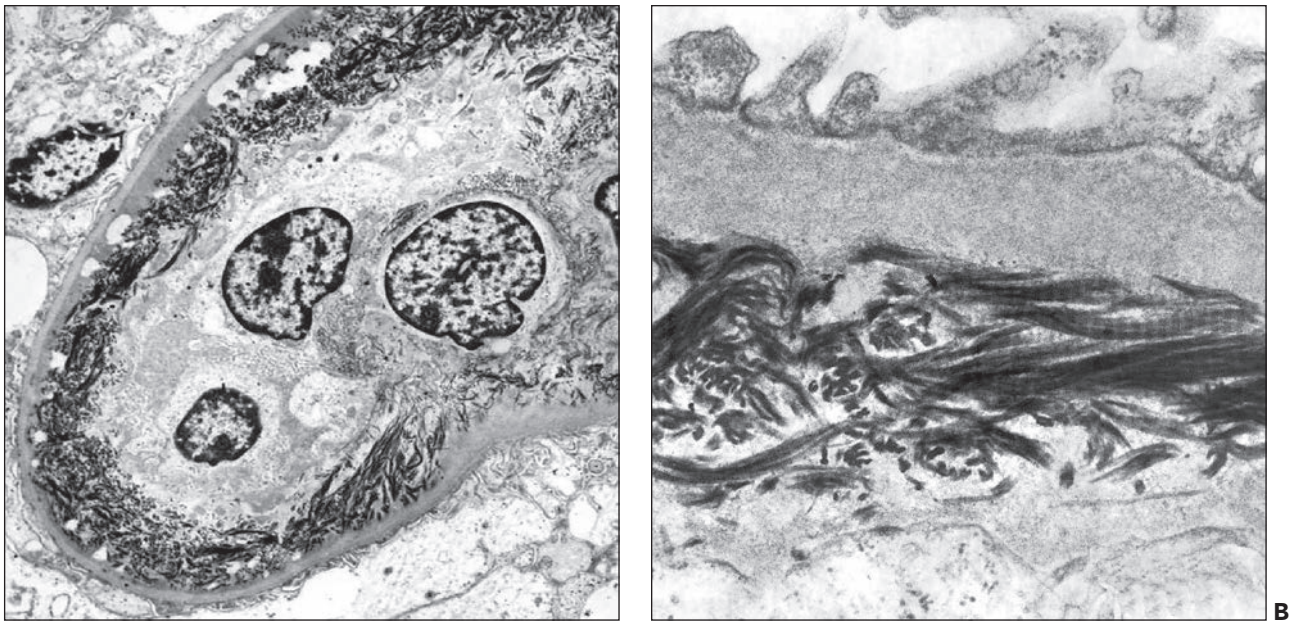


FIGURE 23.16 Collagenofibrotic glomerulopathy. A: Electron micrograph showing peculiar collagen fibrils in expanded mesangial and subendothelial areas. **B:** These fibrils are disorganized and have periodicity. These fibers were proven to be collagen III by immunohistochemistry. (Uranyl acetate and lead citrate; **A:** 5,000×; **B:** 15,000×.)

lack collagen III (89). Collagen III is encoded by a single gene (COL3A1) that has been mapped to chromosome (94). There are two fundamental conceptual ideas or theories regarding the genesis of this disorder. One is that the mesangial cells are deranged and engage in the endogenous production of collagen III that is deposited in the mesangium. Mesangial cells have been shown to contain mRNA for the interstitial collagens (such as collagen III) (95) and have been shown to be able to produce collagen III in vitro (95–97). Myofibroblastic transformation of the mesangial cells endows them with the necessary intracytoplasmic machinery to engage in the excess production of abnormal proteins such as collagen III. Increased procollagen production has been documented in this entity (98).

The second theory is that this disease is a systemic disorder with abnormal metabolism of collagen III (99). This is supported by the fact that there is some evidence of extrarenal manifestations in this entity. The serum concentration of procollagen III peptide is usually markedly increased in these patients and may indeed be a marker for this entity (68,80,100). It has been documented that procollagen III production can be increased in patients with certain kidney disease (101). The extensive subendothelial type III collagen formation in some cases also supports a systemic rather than mesangial origin of precursor procollagen.

Kopp et al. (102) have created a transgenic mouse with increased circulating TGF-B that develops glomerular lesions similar by light microscopy to those seen in collagenofibrotic glomerulopathy in humans, suggesting a role for TGF-B in the pathogenesis of this disorder. However, no staining for collagen III was done, and the typical ultrastructure of fibrils present in collagenofibrotic glomerulopathy was not documented. A better model of the human disease, canine spontaneous autosomal recessive collagen III glomerulopathy, mimics the pathology of the human disease extremely well (103).

Clinical Course, Treatment, and Prognosis

The severity of the clinical manifestations at presentation and rate of progression of the disease process are highly variable. This condition is, at least in a subset of patients, a progressive disease, and ESRD ensues in 5 to 12 years (104). Renal failure has been described within 3 years of diagnosis. In other patients, progression of the renal disease has been documented with increasing proteinuria, hypertension, and renal failure occurring several years after diagnosis (100,105).

No specific treatment is available for this entity. Control of the hypertension and edema is recommended. Steroid therapy is potentially useful in slowing down the progression of the disease process, as it typically suppresses collagen III production in the dermis (106) and decreases procollagen III levels, but the effect on the kidneys has not been examined, and proper clinical trials addressing renal function have not been conducted (92).

Although very few patients have had renal transplants, recurrence in the transplanted kidney has not been documented (104).

ORGANIZED DEPOSITS ASSOCIATED WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus is an autoimmune disease that may involve virtually any organ. The majority of these patients have an immune complex–mediated glomerulonephritis. Typical electron-dense deposits are identified in the various renal compartments. They may be identified in various glomerular locations depending on the class of lupus nephritis present. The electron-dense deposits in lupus glomerulonephritis at times may become organized and create diagnostic

confusion. These organized deposits may be seen in subepithelial, subendothelial, intramembranous, and mesangial locations, as well as extraglomerular sites, including interstitial areas, the peritubular capillary basement membrane, and the juxtaglomerular apparatus (107).

Fingerprints are the most frequent type of organized deposits seen in lupus nephritis. They consist of 2 to 6 regularly stacked, curved, or straight electron-dense bands, 8 to 15 nm in diameter with a center-to-center distance of 19 to 29 nm (108). At very high magnification, cross-striations may be identifiable as a result of lateral projections observed at regular intervals with a center-to-center distance of 10 to 15 nm (Fig. 23.17) (108,109).

Even more rarely, there are distinct tubular structures or fibrils in association with fingerprints or by themselves. The tubules typically have an electron-lucent core and measure 25 to 40 nm in diameter with variable length (Fig. 23.18) (109), and the fibrils measure 8 to 27 nm. These tubular structures must be differentiated from cryoglobulins. Hvala et al. (107) found organized deposits in 20% of biopsies from patients with lupus nephritis.

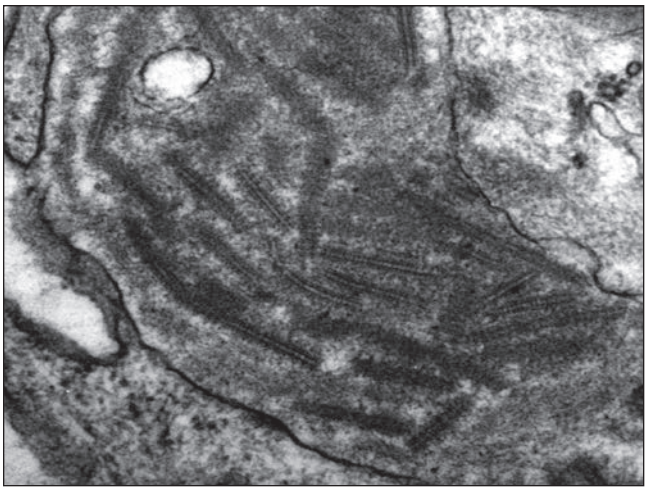


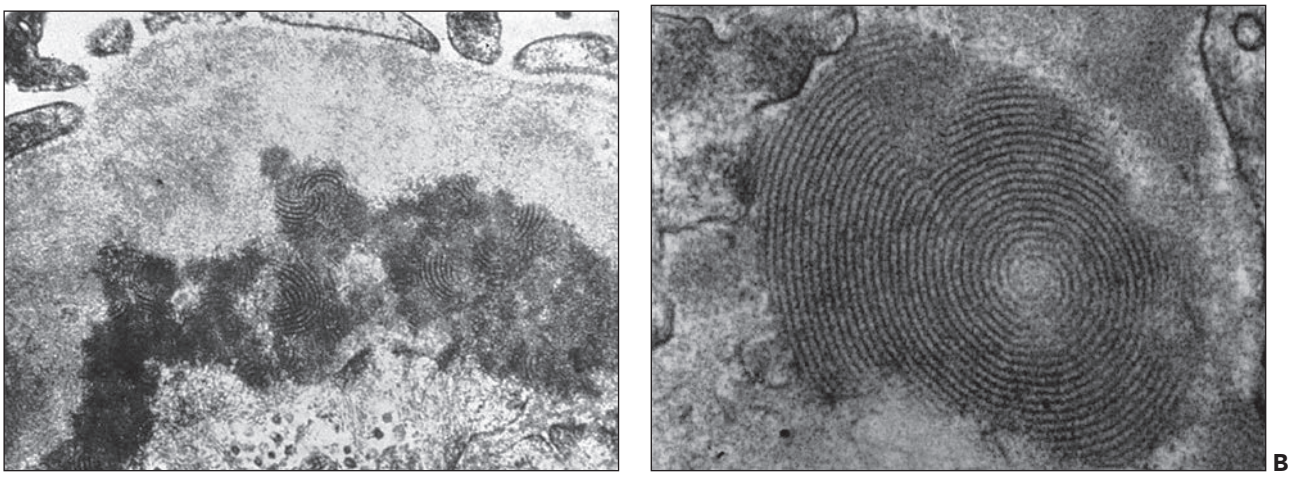
FIGURE 23.18 Lupus glomerulonephritis with organized deposits in the form of microtubular structures. (Uranyl acetate and lead citrate; 20,500×.)

CONFUSING ULTRASTRUCTURAL FINDINGS THAT MAY MIMIC GLOMERULOPATHIES WITH ORGANIZED DEPOSITS

There are a number of structures that may mimic the ultrastructural findings that characterize diseases with organized deposits. These represent artifacts or material that is entirely nonspecific and not diagnostic for a specific disease (9). However, if these are confused with specific structures, an incorrect diagnosis may be reached.

When ultrastructural deposits are related to specific diseases, they generally exhibit relatively uniform structure regardless of where they are located. Deposits that are not indicative of disease-specific entities tend to exhibit marked variability in their appearances (in shape, size, and overall appearance) in different locations (9). While non–disease-associated deposits are most often associated with focal areas exhibiting necrosis and scarring, disease-specific ones are more diffusely present in viable, nonsclerotic tissues. In addition, some of the disease-specific deposits are characteristically associated with distinct immunofluorescence patterns. Some renal diseases are more prone to be associated with nonspecific structured glomerular deposits such as focal segmental glomerulosclerosis and diabetic glomerulosclerosis.

Correlative pathology making use of the combined information obtained from light microscopy, special histochemical stains, immunofluorescence microscopy, and immunohistochemistry is of value in distinguishing nondiagnostic fibrillary matrix material from disease-specific fibrillary deposits and is a must for correct identification of difficult to characterize structures identified by electron microscopy (9). The final assessment of a given case should include a careful evaluation of all the information available. Mistakes are likely to occur in any particular case when one fails to consider the findings in aggregate, findings are not correlated, and conclusions are reached based only on selective evaluation of the data available. There



A **B**

FIGURE 23.17 Lupus glomerulonephritis with fingerprints in immune complex deposits. (Uranyl acetate and lead citrate; **A:** 8500×; **B:** 20,500×.)

are numerous examples of the value of correlative pathology. The matrix is generally silver positive, while disease-specific fibrillary material is usually negative (10). Segmental deposits of insudative/degenerative material in diabetes and focal segmental glomerulosclerosis often react with antibodies to IgM and C3 used in the routine panel of immunofluorescence stains. The fibrils in FGN are very frequently associated with smudgy glomerular deposits of IgG, C3, kappa, and lambda light chains identified by immunofluorescence. Diabetic fibrillosis is typically associated with the typical light microscopic and immunofluorescence microscopy findings of diabetic nephropathy. The clinical information available should also be correlated with the overall biopsy findings. Ultrastructural immunolabeling may also be helpful to link specific antigenic epitopes to ultrastructural correlates, thus clarifying their nature (110).

Altered Mesangial Matrix

In some diseases, the mesangial fibrillary appearance becomes accentuated (Fig. 23.19), and it may be confused with other fibrillary material such as amyloid. This is analogous to diabetic fibrillosis. The fibrillary material in the mesangium is predominantly 4 to 6 nm in diameter and difficult to discern in the normal mesangium. When the fibrils that normally form the mesangium become prominent, as in the case in mesangiolysis, the fibrillary nature of the mesangial matrix becomes noticeable and may be confused with other fibrils such as amyloid. While matrix fibrils most commonly appear organized and display a parallel arrangement, amyloid fibrils are randomly disposed. Both types of fibrils are nonbranching.

In a variety of other conditions, the mesangial matrix may acquire a fibrillary substructure. This may represent biochemically abnormal, structurally deformed, normally or aberrantly synthesized matrix. For example, as noted earlier in this chapter, in a subset of patients with nodular diabetic glomerulosclerosis, the increased extracellular matrix has a conspicuous fibrillary texture (diabetic fibrillosis) (36,61,62). Fibrillary collagen (Fig. 23.20) and precollagen may also be a source of confusion with other fibrillary material and is commonly seen in entities such as focal, segmental glomerulosclerosis in segmentally sclerosed/hyalinized glomerular areas.

In areas of glomerular basement membrane thickening and expanded mesangial matrix caused by a variety of glomerular diseases, including diabetic glomerulosclerosis and glomerulonephritis, there may be membrane fragments, lipid vacuoles, and cellular debris (so-called matrical lipid debris) (111–113). Immunoelectron microscopy demonstrates a frequent association of complement component with these structuures, especially C3d and C9 (113). All these can be confusing to the inexperienced eyes and even troublesome to experienced renal pathologists.

Glomerular Sclerosis

Glomerular sclerosis may be associated with the deposition of banded fibrillary collagen and/or nonfibrillary collagen, as well as precollagen. In this setting, precollagen can also be a source of confusion, as it lacks the typical banding pattern of mature collagen. Segmental glomerular sclerosis represents more of a problem than global glomerular sclerosis. It is difficult to identify specific diagnostic features in globally sclerosed/hyalinized glomeruli and in segmentally sclerosed glomeruli where areas with hyalinosis and cellular debris are common findings. However, when the pattern of sclerosis is placed in the context of the overall findings by light, immunofluorescence, and electron microscopy, the glomerular sclerosis may have characteristic features that suggest the origin for the scarring, for

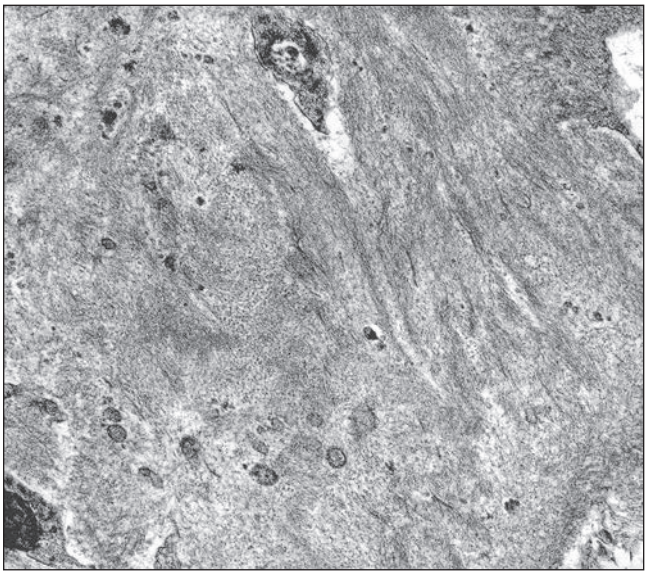


FIGURE 23.19 Accentuated mesangial matrix fibrillary appearance. (Uranyl acetate and lead citrate; 54,800×.)

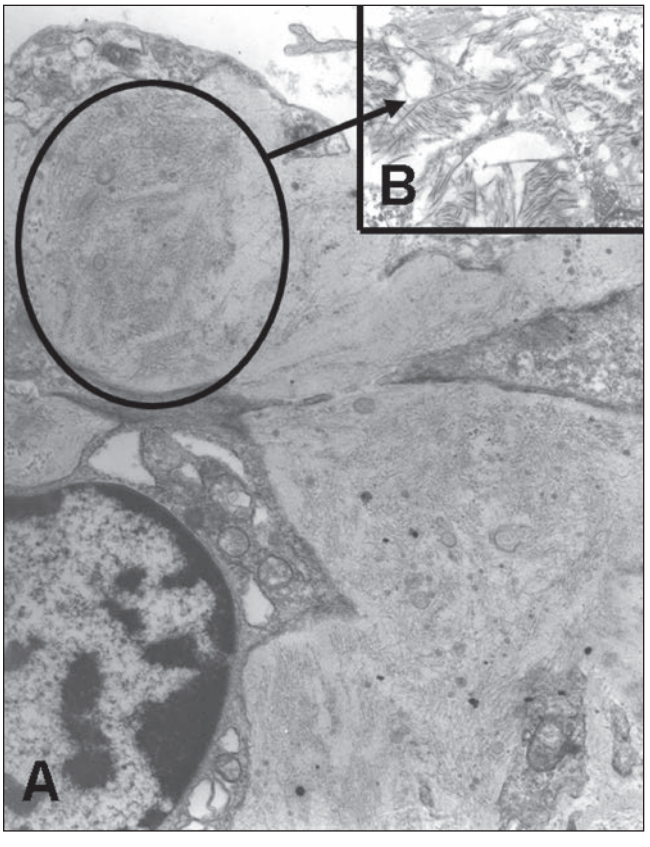


FIGURE 23.20 Banded collagen fibers in expanded mesangial area. (**A,B:** Uranyl acetate and lead citrate; 10,000×.)

example, focal segmental glomerulosclerosis, arterionephrosclerosis, diabetic glomerulosclerosis, or the sclerotic phase of a glomerulonephritis.

Cellular Debris

Cellular debris in the mesangium may have a variety of ultrastructural appearances difficult to separate from diagnostically specific structures. Different cellular components, mostly organelles, may fragment, appear outside of the cell bodies, and create structures identifiable by electron microscopy (Fig. 23.21). This debris may be associated with complement components detectable by immunoelectron microscopy (113). This may be a source of confusion with diagnostic entities such as immunotactoid glomerulopathy (114). The mesangial matrix in nodular glomerulosclerosis is particularly prone to this.

Intracellular Fibrils

Intracytoplasmic fibrils may sometimes appear to be extracellular and create confusion if the cellular outlines become ill defined as a consequence of pathology (i.e., necrosis) or poor fixation/autolysis. This occurs in particular in cases where the cell membranes are not well preserved and it becomes difficult to separate intracellular from extracellular compartments (9).

Fibrin Tactoids

On light microscopy, this material generally appears vaguely fibrillary. Fibrin is characteristically red on the trichrome stain. Phosphotungstic acid-hematoxylin stain may be useful to confirm the presence of fibrin, which stains blue. Ultrastructurally, fibrin tactoids are typically observed in clusters and appear as curved, parallel, osmiophilic deposits displaying cross-striations with a periodicity of about 23 nm (Fig. 23.22) that is often detectable (9), but in some cases, the typical substructure of fibrin is not clearly apparent. It is in these situations that confusion with fibrillary material such as amyloid may occur.

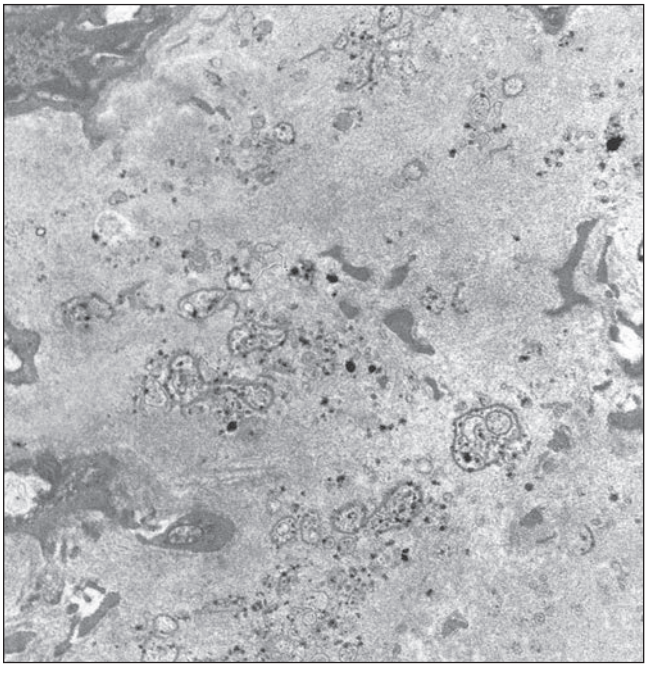


FIGURE 23.21 Debris in the mesangium. Microspherules and cell membrane-like fragments in the expanded mesangial matrix. (Uranyl acetate and lead citrate, 22,500×.)

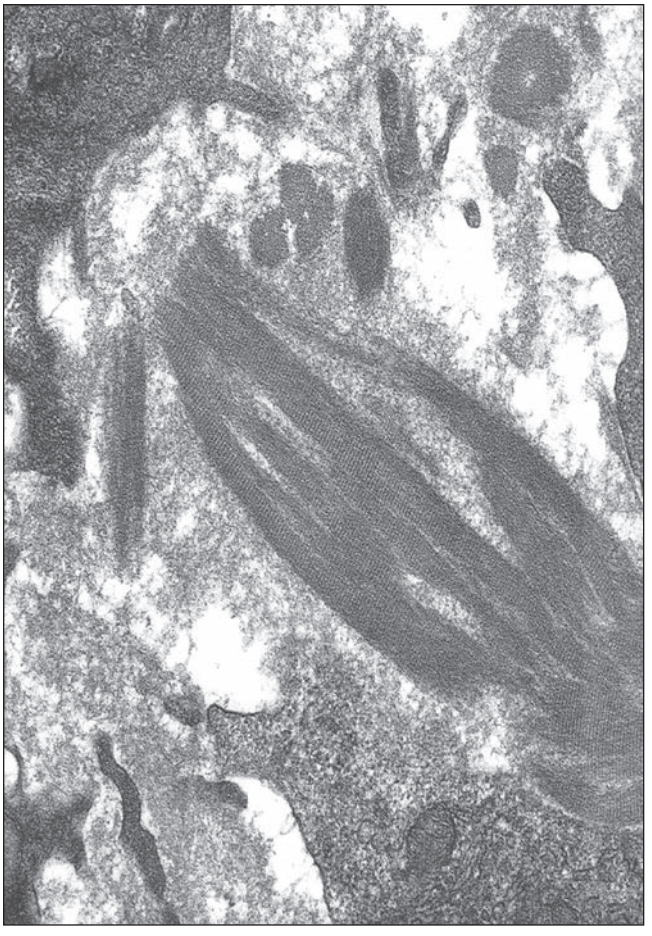


FIGURE 23.22 Fibrin tactoids with typical periodicity. (TEM, uranyl acetate and lead citrate, 17,500×.)

Ultrastructural Artifacts

Artifacts may be difficult to accurately characterize. Artifacts are most prevalent in specimens mishandled as a result of delay or improper fixation of the samples or retrieval from paraffin or frozen blocks. Prompt and adequate fixation of the renal biopsy samples eliminates the majority and most common artifacts. Use of the proper fixative for ultrastructural evaluation and sectioning of the samples into small 1-mm cubes allows for adequate penetration of the fixative, and appropriate fixation generally avoids artifacts. Overheating of tissues while processed for light microscopy results in marked tissue changes, especially if specimens are taken from paraffin blocks and reprocessed for electron microscopy.

Artifacts can assume myriads of ultrastructural appearances. Small microspherical particles (Fig. 23.23A) can be particularly troublesome, as they may mimic a number of structures such as viral particles (virions) and cross-sectioned

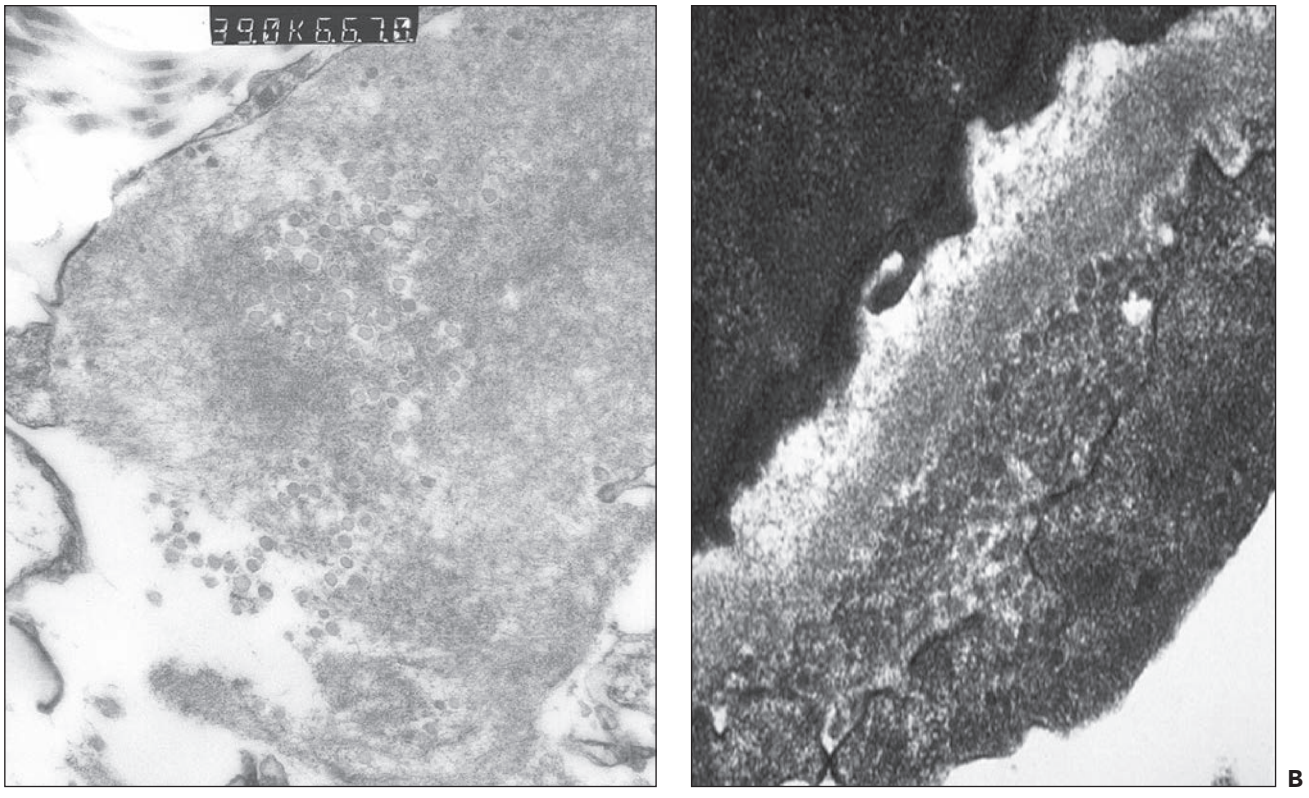


FIGURE 23.23 Microspherical particles. A: Microspherical particles in the mesangium. **B:** Microspherical particles in subepithelial deposits of membranous glomerulonephritis. (Uranyl acetate and lead citrate; **A:** 65,000×; **B:** 22,500×.)

profiles of cryoglobulins. These should not be confused with microspherical particles that are not artifacts but rather are a distinctive feature of a disease process. For example, in a small number of membranous nephropathy cases, microspherical particles are seen within the immune complexes along peripheral capillary walls (Fig. 23.23B). These microspherical particles are not artifacts and typically are present in repeat biopsies from the same patient. The epidemiology and clinical course of these patients are similar to other matched membranous nephropathy cases. Kowalewska et al. examined 864 cases of membranous nephropathy and found seven of these with microspherical particles associated with immune complexes along peripheral capillary walls (0.7% of all membranous cases). He added to his study seven similar cases. One of the cases occurred posttransplant. All of the patients presented with proteinuria (majority in the nephrotic range). Associated systemic conditions included lupus, diabetes mellitus, stem cell transplantation, Sjögren syndrome, pregnancy, and renal transplant (114). Similar particles have also been reported in focal, segmental glomerulosclerosis (115).

Delineation between intra- and extracellular compartments may become blurred, as cellular membranes may disappear or fragment when samples are not handled appropriately. Intracellular material may appear to be extracellular. Extracellular matrix artifacts can mimic structured deposits such as cryoglobulins or deposits seen in immunotactoid glomerulopathy (116), among others.

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CHAPTER 24

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Pyelonephritis and Other Direct Renal Infections, Reflux Nephropathy, Hydronephrosis, Hypercalcemia, and Nephrolithiasis

PYELONEPHRITIS AND OTHER INFECTIONS

Introduction, Terminology, and Historical Perspective

Pyelonephritis is a bacterial urinary tract infection (UTI) affecting the kidney parenchyma, calyces, and pelvis. It occurs in two forms, *acute* and *chronic*, and may be found with or without obstruction of the urinary tract (*obstructive and nonobstructive pyelonephritis*).

While bacteria cause immunologic glomerular injury and glomerulonephritis, this topic is not discussed here (see Chapter 10).

The infecting organisms are thought to be of intestinal origin and reach the kidney from the lower urinary tract by an ascending route. Rarely, bacteria in the bloodstream may colonize the kidney (1). Most UTIs are caused by Gram-negative enteric organisms and can be symptomatic or asymptomatic, classified as uncomplicated or complicated (Table 24.1). Uncomplicated UTIs occur in otherwise healthy individuals. Women are affected more than men, and the most common bacteria are *Escherichia coli* (*E. coli*) (80%) (1–4). Uncomplicated UTI is characterized by frequency, urgency, dysuria, or suprapubic pain. Acute nonobstructive pyelonephritis occurs in the same population that experiences acute uncomplicated UTI and is characterized by costovertebral angle pain and tenderness, often with fever. Complicated UTIs occur in immunocompromised patients, those undergoing catheterization, or those with functional or anatomical abnormalities and involve either the bladder or the kidneys. These infections are typically polymicrobial and include *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, among others. The infectious landscape changes depending on the patient's age and the presence of comorbid conditions such as diabetes, spinal cord injury, and immunologic abnormalities. Even though *E. coli* accounts for the majority of UTIs, in the elderly, polymicrobial infections with Gram-positive organisms are present in approximately one third of cases. Uncomplicated UTIs in children are most commonly caused by *Enterobacteriaceae* and may predispose them to adult disease (5,6). UTIs can have serious complications such as premature delivery in pregnant women, sepsis in the elderly, and renal scarring in children (1–6).

TABLE 24.1 Clinical syndromes of acute pyelonephritis

Uncomplicated pyelonephritis
in women
Subclinical pyelonephritis
Acute symptomatic pyelonephritis
Pyelonephritis in pregnant women
Recurrent pyelonephritis
In men (<50 years old)
Subclinical pyelonephritis
Acute symptomatic pyelonephritis
Complicated pyelonephritis
structural or functional abnormalities
Prostate disease (benign prostatic hyperplasia, prostatitis)
Obstruction
Calculi
Neurologic disease
Vesicoureteral reflux
Urologic manipulation
Intubated drainage (bladder catheter)
Urinary instrumentation (cystoscopy)
Renal transplantation
Underlying disease
Diabetes
Renal failure
Immunosuppressed or immunodeficient
Cystic renal disease

In 2007, UTIs accounted for 8.6 million ambulatory care visits in the United States (7,8). About 25% of affected women develop recurrent UTI within 6 to 12 months and can be either a "relapse" or "reinfection." Relapse is defined as a recurrent UTI occurring after therapy and is due to persistence of the pretherapy bacteria. "Reinfection" is recurrent UTI with an organism originating outside the urinary tract, either a new bacterial strain or a strain previously isolated that persisted in the colonizing flora of the gut or vagina. Relapse of UTI requires appropriate management and prophylactic treatment according to clinical practice guidelines (9,10). The kidneys may be involved in such cases, increasing the risk of developing hypertension or renal failure.

Infecting organisms may also reach the kidney via blood-borne invasion. The most common organism infecting the kidney by this route is *Staphylococcus aureus*. This infection consists of vast numbers of minute abscesses scattered throughout the parenchyma, particularly the cortex. The terms *multiple cortical abscesses*, *diffuse suppurative nephritis*, and *diffuse bacterial nephritis* are used for this picture. *Staphylococcus aureus* has the ability to localize, proliferate, and incite an acute inflammatory reaction in an unobstructed kidney. In contrast to ascending infection, in blood-borne infections, minimal inflammatory changes are found in the pelvis and calyces; those that are present are secondary to the cortical infection.

Acute UTIs and the causative role of *E. coli* were established over 100 years ago; the terms *cystitis* and *pyelitis* were introduced at that time (11,12). Thiemich's important observation that the renal parenchyma was frequently infected in what had previously been regarded as pyelitis gave birth to the concept of acute pyelonephritis (13). A full appreciation of the side effects of acute kidney infection came later and not until 1917 when Löhlein defined the clinical and pathologic features of the pyelonephritic kidney in three young women who died of uremia, two of them with hypertension (14).

In the context of pyelonephritis, it is important to define the term *bacteriuria*. *Bacteriuria* simply means that there are organisms in the specimen of urine tested but does not necessarily imply infection of the kidney. It may be found in patients with and without renal involvement. Furthermore, *bacteriuria* may result from contamination during collection. As might be expected, the urine passed at the beginning of micturition is most likely to be contaminated. Midstream specimens of urine are less likely to be contaminated and are therefore used to determine whether the bladder urine is infected. Suprapubic aspiration of urine avoids contamination by the urethral flora. Although this is an invasive procedure, it is frequently employed in infants. To distinguish between genuinely infected urine and contaminated urine, it is necessary to perform quantitative bacterial counts. Kass (15) demonstrated that bacterial counts of more than 100,000 colony-forming units (cfu) per milliliter of urine usually represent genuine infection. This is referred to as *significant bacteriuria*. Kass was careful to point out that a lower figure could indicate true infection under such conditions as rapid urinary flow, when urine pH is low or when bacteriostatic drugs are being used. To these can be added other factors, but over the years, the figure of 100,000 cfu/mL of urine has provided a workable basis for the determination of significant bacteriuria. A figure of 100 cfu/mL has been proposed for the specific instance of women with acute dysuria and frequency (16).

In certain patients, particularly women, significant numbers of bacteria are found in the urine on routine testing despite the fact that there are no clear clinical manifestations. The name *asymptomatic bacteriuria* or *covert bacteriuria* is given to this

situation. There is an increased prevalence of asymptomatic bacteriuria in older patients; in men, it is commonly caused by prostatic enlargement and loss of bactericidal activity of prostatic secretions (17). Poor bladder emptying due to uterine prolapse is considered important in women (18,19). Neuromuscular disease, increased instrumentation, and catheter use contribute in both sexes. Asymptomatic bacteriuria, or asymptomatic UTI, is defined by the U.S. Preventive Services Task Force as isolation of a specified quantitative count of bacteria in an appropriately collected urine specimen obtained from a person without symptoms or signs referable to urinary infection (20). Asymptomatic bacteriuria is due to bacteria that lack virulence factors (discussed under pathogenesis).

UTI associated with catheter use has caused considerable confusion as to the appropriate management and/or treatment in the elderly, and several authoritative guidelines are published recently to reinforce understanding of the definition of asymptomatic bacteriuria and symptomatic UTIs (Table 24.1) (21–24).

Lastly, the term "pyuria" refers to the presence of increased numbers of polymorphonuclear leukocytes in the urine and constitutes evidence of an inflammatory response in the urinary tract (20).

Distinct types of chronic pyelonephritis such as xanthogranulomatous, emphysematous pyelonephritis, and malakoplakia are discussed separately because of their unique pathology and clinical presentation.

Acute Pyelonephritis

Clinical Presentation

In a classic case of lower UTI in the adult, the patient presents with rapid onset of chills, fever, lumbar tenderness, dysuria, and urinary frequency. Hypertension is usually absent. Symptoms such as frequency, urgency, suprapubic discomfort, and flank pain should lead to screening. Young children may present with nonspecific symptoms, such as poor feeding, vomiting, irritability, jaundice (in newborns), or fever alone, and a broader approach to screening may be appropriate (2,5). Acute renal failure occasionally occurs particularly in the elderly (25). Although bacteremia causes chills, it seldom causes more serious side effects, such as disseminated intravascular coagulation. The urine contains organisms in excess of 100,000 cfu/mL, and white blood cells (pyuria) and white blood cell casts are present in the sediment. White blood cell casts are significant, implying inflammation in the kidney. Proteinuria may or may not be present and is seldom heavy. Macroscopic or microscopic hematuria may develop as a result of small hemorrhages in the renal pelvis or bladder.

The clinical diagnosis of acute pyelonephritis is relatively crude and imprecise. It is often impossible to distinguish between acute pyelonephritis and infections confined to the lower urinary tract on purely clinical grounds (2). Various ancillary laboratory tests including tests for impaired urine concentration, increased serum concentration of C-reactive protein, and estimation of the lactic dehydrogenase concentration in the urine are not very useful. Urine dipstick for leukocyte esterase and nitrites and standard microscopy on a centrifuged specimen are very helpful; the so-called enhanced urinalysis that combines high-power microscopy with a hemacytometer and Gram stain of unspun urine for organisms have high predictive value (95%) (5). These tests are often complemented by various imaging techniques to add diagnostic precision. Ultrasonography (increased renal size), intravenous urography (renal enlargement with reduced nephrogram), and radionuclide methods using gallium 67 citrate and iodine 131 Hippuran (defective uptake) have been used with varying degrees of success

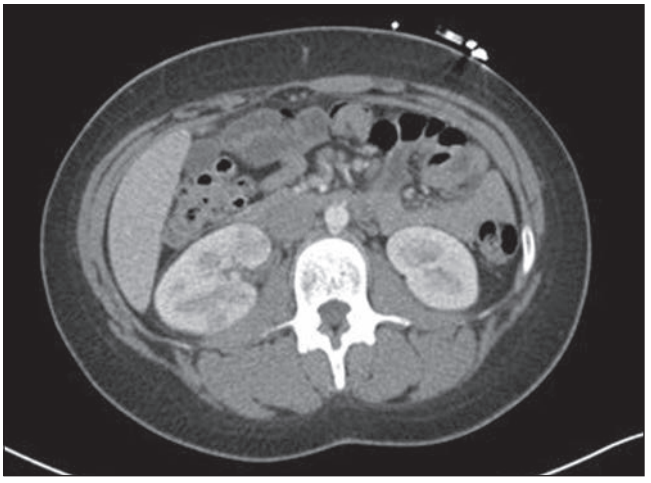


FIGURE 24.1 Contrast-enhanced transverse CT shows multiple wedge-shaped areas of lower attenuation with effacement of the corticomedullary junction in the right kidney. These findings are highly suggestive of pyelonephritis. (Courtesy of Cary Siegel, Mallinckrodt Institute of Radiology, St. Louis, Missouri.)

(26–28). Computed tomography (CT) is currently the preferred method for assessing the extent of parenchymal involvement and possible complications of pyelonephritis such as parenchymal or perinephric abscess or emphysematous pyelonephritis, which are clinically difficult to diagnose in some cases and in atypical infections (26–28). In addition, CT can provide physicians with much more information about underlying abnormalities, such as stones, and congenital urologic anomalies. Unenhanced CT is excellent for identifying stones, gas, or obstruction; contrast-enhanced CT is better for acute pyelonephritis, which usually manifests as wedge-shaped (streaky) areas of low enhancement extending from the papilla to the renal cortex. The striated enhancement represents tubular obstruction by inflammatory cells and the associated edema of the renal parenchyma (Figs. 24.1 and 24.2).

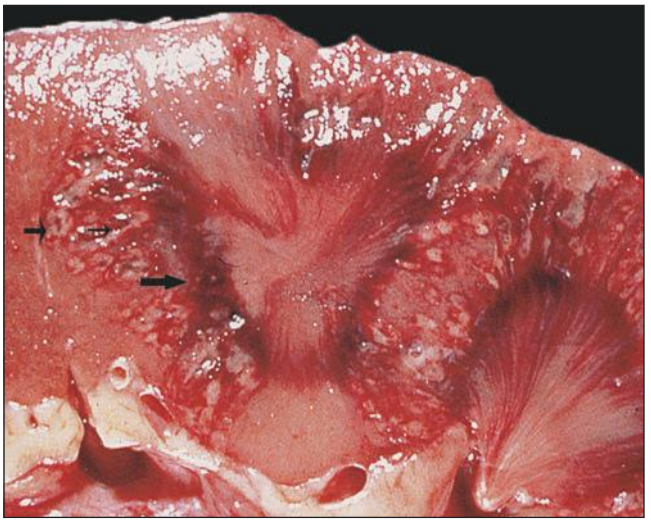


FIGURE 24.2 Acute pyelonephritis. Cortical abscesses are apparent and straight yellow streaks (*thin arrows*) and hyperemia in the medulla (*thick arrow*).

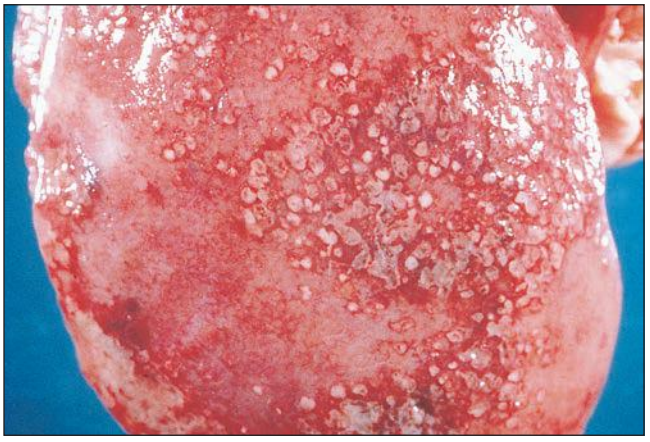


FIGURE 24.3 Acute pyelonephritis. Cortical abscesses produce discrete or confluent, raised, yellowish-white, rounded nodules with surrounding hyperemia on the subcapsular surface.

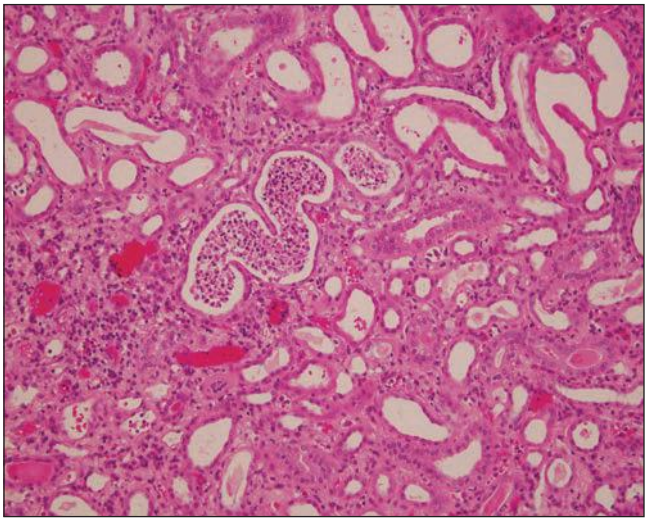


FIGURE 24.5 White cell casts in acute pyelonephritis. (H&E; ×400.)

Gross Pathology and Light Microscopy

Acute pyelonephritis seen by the pathologist grossly is limited to surgically resected obstructed kidneys. *Obstructive acute pyelonephritis* presents as an enlarged kidney with a bulging cut surface. The cortex contains whitish areas of acute infection. Between these are scattered, small, discrete, whitish-yellow abscesses with a hemorrhagic rim. These small abscesses, measuring up to several millimeters in diameter, are seen particularly well on the subcapsular surface (Fig. 24.3). In some cases, almost the entire cortex is white and swollen. The pelvis and calyces are dilated. The mucosal surfaces are often congested, with thickening of the pelvic wall. In cases of severe obstruction, the renal parenchyma may be thinned with blunted papillae and the pelvis filled with pus. This situation is referred to as *pyonephrosis* (Fig. 24.4).

In addition to the cortical changes, the medulla shows characteristic straight, whitish-yellow streaks corresponding to pus-filled collecting ducts (see Fig. 24.2). Papillary necrosis may be present, particularly in diabetic patients with severe, often terminal, acute renal infection. Secondary involvement of the kidney occurs via a hematogenous route. Regardless of whether it is the *obstructive* or *nonobstructive* type, the histology of acute infection is similar. Tubules are extensively destroyed by acute, neutrophil dominant, inflammation (Fig. 24.5). Neutrophils may also fill the medullary collecting ducts. Acute inflammatory changes are seen in the pelvic and calyceal epithelium (Fig. 24.6). They are generalized in obstructive forms but restricted to the involved calyceal systems in nonobstructive forms. Papillary necrosis may be seen in severe terminal renal infections with obstruction and diabetes (2,3). An important feature of acute nonobstructive pyelonephritis is the way large areas of parenchyma are spared from infection.

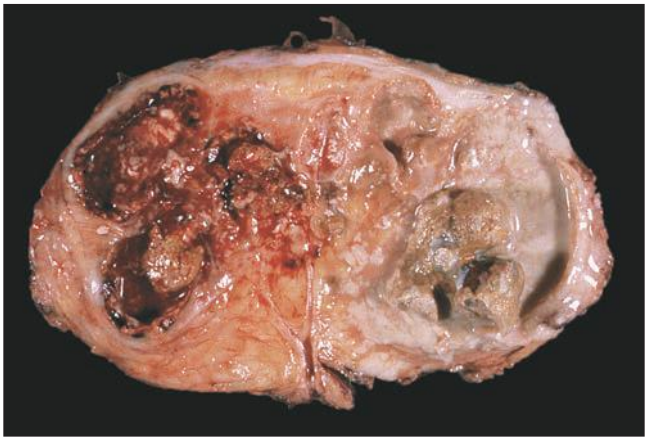


FIGURE 24.4 Pyonephrosis. The kidney is converted into a pus-filled sac, with little identifiable parenchyma. The mucosa of the collecting system is focally hemorrhagic and covered by creamy exudate; it contains several calculi.

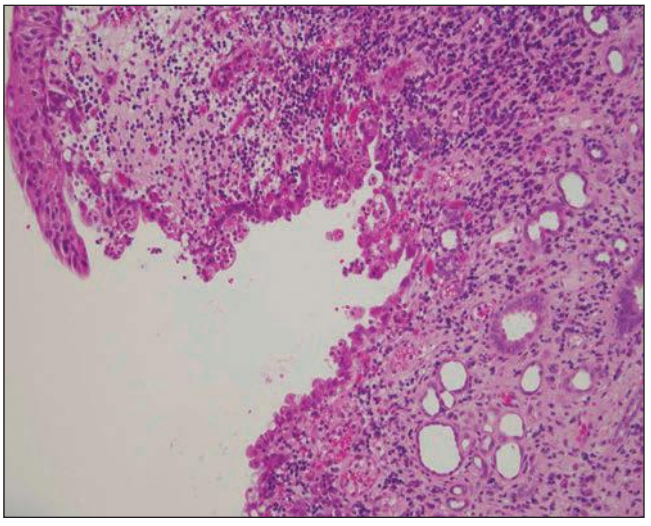


FIGURE 24.6 Acute pyelitis; neutrophils erode the lining epithelium forming microabscesses. (H&E; ×200.)

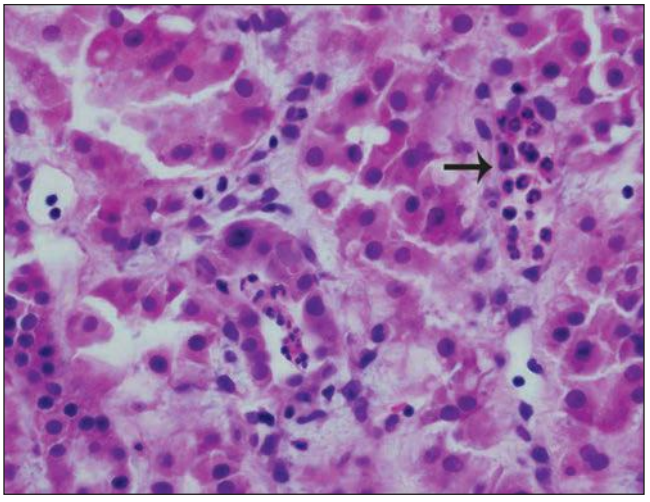


FIGURE 24.7 In acute pyelonephritis, neutrophils appear first in peritubular capillaries (*arrow*). (H&E; ×400.)

Initially, the inflammatory exudate consists of neutrophils, first appearing in the intertubular capillaries (Fig. 24.7). This is followed by capillary wall rupture, with leakage of fluid and cells into the interstitium (Fig. 24.8) (29). Chronic inflammatory cells, such as macrophages, lymphocytes, and plasma cells, appear within a few days of the start of infection as neutrophils disappear fast (30,31). Glomeruli are surprisingly resistant to damage (Fig. 24.8). Although some glomeruli are secondarily involved by inflammation—invasive glomerulitis—the vast majority remain unscathed (32).

Small interstitial capillaries are also involved in acute pyelonephritis as mentioned earlier; they are occluded by leukocyte plugs. Larger vessels contain acute inflammatory cells adhering to the endothelium. Endothelial cell necrosis, vascular wall fragmentation and remodeling around the endothelium, adherent degranulated neutrophils containing phagocytosed bacteria can be seen ultrastructurally. The perivascular interstitial matrix is filled with collagen fibrils or fibrin deposits. Reparative changes including capillary neovascularization are not infrequent (29). In addition to small-vessel damage, the renal vein or artery may undergo thrombosis in severe acute pyelonephritis (33).

A diagnosis of pure acute pyelonephritis in native or allograft renal biopsies is currently infrequent. Diagnostic histopathologic findings are similar to obstructive pyelonephritis in surgically resected kidneys and consist of significant interstitial inflammatory infiltrate likely occupying about 50% of biopsy surface area and intratubular white cell casts. These findings in the appropriate clinical scenario, such as fever and positive urinary cultures, pose no diagnostic difficulty. Problems arise when tubular inflammation is focal, blood or urine cultures are negative, patients are partially treated prior to biopsy, or atypical infectious microorganisms underline the process. Focal intratubular neutrophils or white cell casts can be found in other diseases, for example, acute interstitial nephritis, cast nephropathy, and acute tubular necrosis (ATN). In allograft renal biopsies, the differential diagnosis includes tubulointerstitial rejection and acute allergic—drug-induced—interstitial nephritis (34–36). Acute cellular rejection is mediated by mononuclear lymphocytes infiltrating the tubular epithelium. Neutrophils, if present, are rare and only usually do not form tubular casts. Currently, most kidney transplant recipients are preemptively treated with antibiotics, usually vancomycin, to prevent UTIs; thus, acute pyelonephritis is an infrequent complication in this patient population (36).

A particularly challenging situation is excluding ATN associated with neutrophilic infiltrates. For example, white cell casts may be present in renal biopsies from patients presenting with acute renal failure and negative blood/urine cultures; multifocal ATN is the only finding in some cases. The role of infection in causing acute renal failure and presumed ATN has been addressed in the literature, but renal biopsy findings are not adequately documented (37). ATN is associated with an inflammatory response that includes monocyte/macrophage

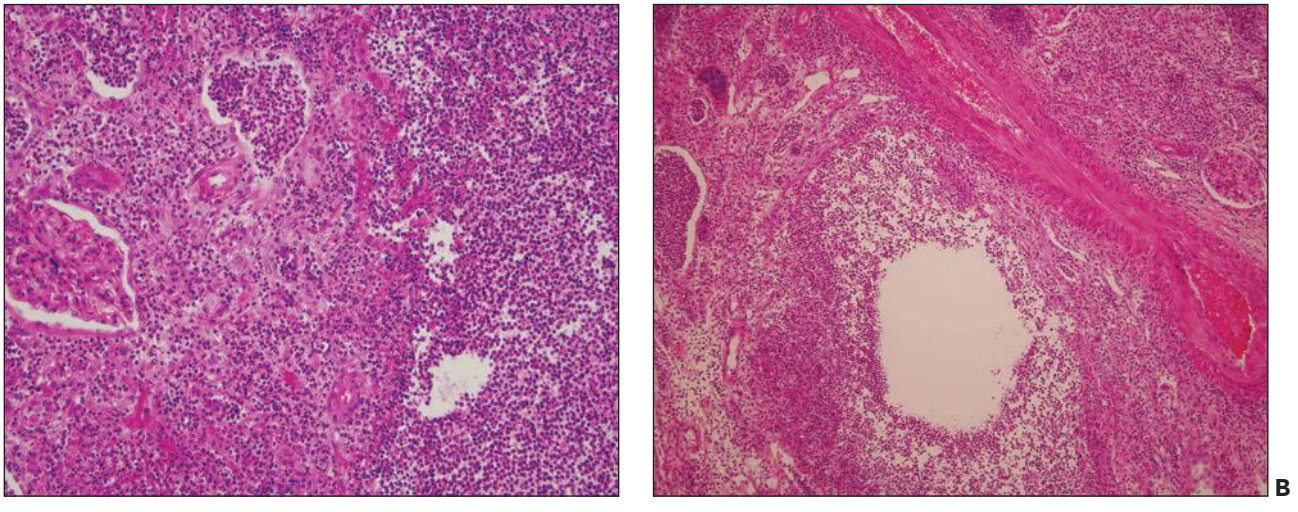


FIGURE 24.8 **Acute pyelonephritis. A:** Pools of neutrophils destroy tubules; glomeruli are remarkably unaffected. **B:** Abscess formation. (H&E; ×200.)

and neutrophil recruitment to the kidney, which may worsen renal injury, but there is considerable controversy on the pathology of ATN in humans compared to animal models (38). Recent studies suggest that interstitial injury activates innate immunity and the inflammasome, a new concept to explain inflammatory responses in kidney disease in various conditions including ATN and obstruction (39,40). Innate immune response in the context of kidney infection is discussed further under pathogenesis of pyelonephritis.

Specific Forms of Acute Pyelonephritis

Diffuse Suppurative Pyelonephritis

This lesion is caused by blood-borne infection of the kidney, as opposed to ascending infection. It is typically caused by *S. aureus*, an organism that can localize in the kidney without obstruction. It may also be seen with *E. coli* bacteremia, but only when there is obstruction to urinary outflow. The source of infection is often nosocomial. *Staphylococcus aureus* is the most common organism. Immunosuppressed patients are particularly vulnerable to staphylococcal infections (34,35). Moreno et al. (34) studied 75 episodes of bacteremia or fungemia in renal transplant recipients. They found that the kidney and urinary tract was the site of infection in 21 cases (28%), and staphylococci were the most common infectious organisms. Patients presented with fever, lumbar pain, symptoms of lower UTI, and renal failure (34).

Grossly, the kidneys are enlarged. The subcapsular surface is studded with numerous whitish-yellow abscesses, often with red rims. The abscesses vary in size; some are as small as a pinhead, but others may measure up to half a centimeter across (Fig. 24.9). Abscesses are usually discrete, but some are confluent. The cut surface bulges because of the accompanying interstitial edema; some are rounded and similar to those seen on the subcapsular surface, but others are wedge shaped with the apex pointing inward. Most abscesses are in the cortex, although some are in the medulla, particularly the outer part. Whitish streaks are often seen in the medulla, representing pus-filled collecting ducts. The pelvis and calyces are not usually dilated. Both kidneys are affected and equally enlarged.

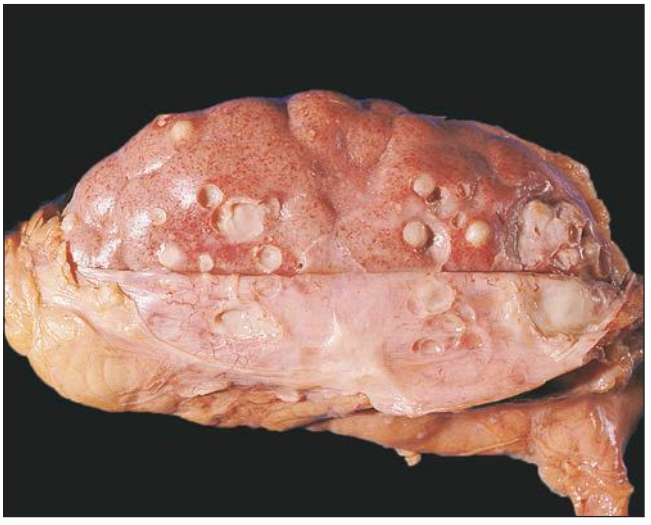


FIGURE 24.9 Diffuse suppurative nephritis. The subcapsular surface shows numerous discrete and focally confluent, whitish-yellow abscesses of variable size.

Histologically, abscesses consist of large numbers of interstitial neutrophils, with extensive destruction of tubules, particularly the proximal convoluted segments (Fig. 24.10A). Glomeruli, arteries, and arterioles are usually undamaged, although microabscesses may occasionally be seen in the glomeruli. Undamaged tubules may be filled with neutrophils, accounting for the linear streaking seen grossly in the medulla. Organisms are readily evident (Fig. 24.10B). In contrast to acute pyelonephritis, there are no, or only a few, inflammatory cells beneath the calyceal and pelvic epithelium (35). Immunocompromised patients may have a modified response

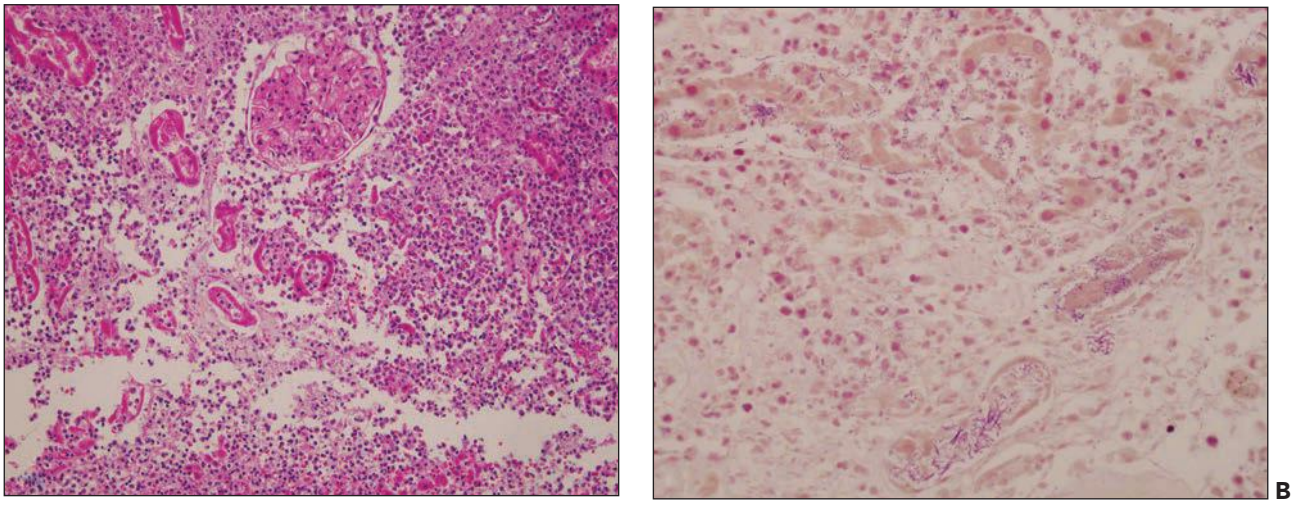


FIGURE 24.10 Suppurative pyelonephritis from a 68-year-old man with diabetic end-stage kidney disease. A: Massive neutrophil influx obscures and destroys proximal tubules. (H&E; ×200.) **B:** Gram-negative bacteria admixed with neutrophils in tubules and the interstitium. (Gram stain × 400.)

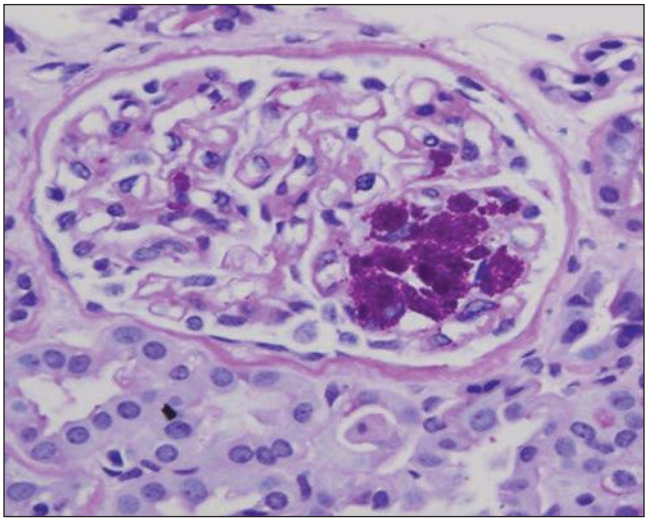


FIGURE 24.11 Acute pyelonephritis from an immunocompromised patient with *Tropheryma whippeli* infection. PAS-positive organisms are found within the glomerular tuft. (PAS; ×400.)

to hematogenously spread infection to the kidney. For example, the case shown in Figure 24.11 is from a 68-year-old man with history of weight loss, fatigue, weakness for 2 years, history of colon cancer, and documented blood infection. The glomeruli are filled with periodic acid-Schiff (PAS)–positive rod-shaped *Tropheryma whippeli* bacteria, better seen by electron microscopy (Fig. 24.12). Acute pyelonephritis was evident in the surrounding renal parenchyma, but the glomeruli are devoid of inflammation in spite of the presence of bacteria.

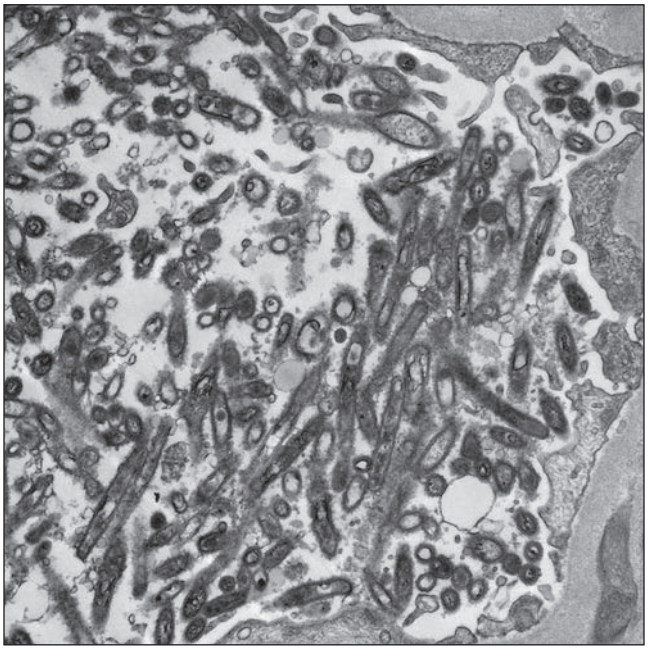


FIGURE 24.12 Transmission electron micrograph demonstrates the rod-like *Tropheryma whippeli* organisms within the glomerulus. (Courtesy of Carrie Phillips, Indiana University School of Medicine, Indiana.)

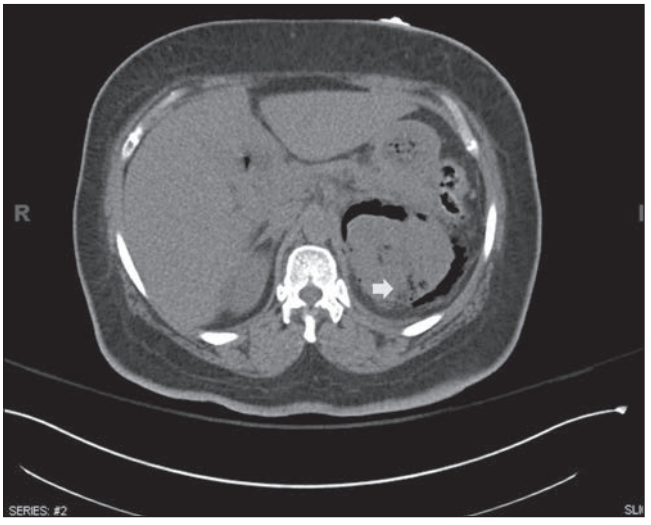


FIGURE 24.13 Emphysematous pyelonephritis. The patient was a 58-year-old woman with history of diabetes who presented with flank pain. CT coronal reconstruction shows gas in the parenchyma of the left kidney and extensive perinephric gas (*arrow*). (Courtesy of Sanjeev Bhalla, Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, MO.)

Emphysematous Pyelonephritis

Emphysematous pyelonephritis consists of a severe suppurative infection of the kidney accompanied by gas formation in the pelvicalyceal region (emphysematous pyelitis), the kidney parenchyma, and sometimes in the perirenal tissue. Parenchymal abscesses and infarction, with gas formation in necrotic areas or papillary necrosis and vascular thromboses, are common (Figs. 24.13 and 24.14). Obstruction was recorded in 40% of cases. Ninety-five percent of patients with emphysematous pyelonephritis have diabetes (42). Drug abuse,

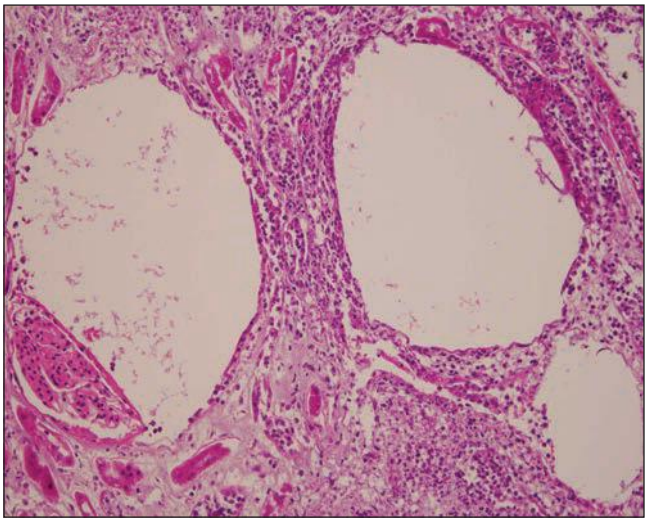


FIGURE 24.14 Emphysematous pyelonephritis. The patient was a 68-year-old diabetic woman. Gas formation in necrotic tissue produces circular spaces resembling pulmonary emphysema. (H&E; ×200.)

neurogenic bladder, alcoholism, and anatomic anomalies have also been found in association with this disease (43). Women are affected more often than are men, with a mean age in the sixth decade. Typically, only one kidney is involved, usually the left (3). Rarely, both kidneys are affected (3). Emphysematous pyelonephritis is associated with a 21% mortality rate (44). Patients may have nonspecific clinical symptoms including chills, fever, flank pain, nausea, vomiting, abdominal pain, and pyuria. Patients may initially present with thrombocytopenia, acute renal failure, disturbance of consciousness, and shock, which are risk factors for poor outcome and mortality (44). *Escherichia coli* is the most common organism encountered, but others, such as *Klebsiella pneumoniae*, *Enterobacter* spp., *Proteus mirabilis*, *Candida* spp., and *Cryptococcus neoformans*, have been described. Diagnosis can be made by CT, which also provides information for classifying the extent of the intrarenal and extrarenal disease, which has prognostic and therapeutic importance (2,3,26,27,44). Emphysematous pyelonephritis is a very serious condition that requires prompt and energetic treatment. Operative and nonoperative treatment with antibiotics is currently employed avoiding nephrectomy. The allograft kidney is rarely affected with only about 20 cases of emphysematous pyelonephritis reported in the literature (43). A new radiologic classification is proposed taking into account the extent of gas accumulation in the kidney (class 1 to 4) to help guide appropriate and timely management of patients (44). The pathogenesis of the condition is not clear, but many features are similar to those described in *S. aureus* infection, suggesting a bloodborne infectious etiology. Four factors involved in pathogenesis include gas-forming bacteria, high tissue glucose, impaired tissue perfusion, and a defective immune response (44,45,62). Successful medical therapy is possible in some cases (45).

Sepsis and kidney injury

Sepsis is currently defined as a systemic inflammatory response associated with a confirmed infection. There appear to be two phases in response to sepsis. First, a proinflammatory response results in hypotension and organ dysfunction. This is followed by an anti-inflammatory response causing immune depression (46). The mechanism of kidney injury in sepsis is multifactorial. Hemodynamic instability, diffuse intravascular coagulation, inflammatory mediators, and tubular obstruction secondary to tubular cell death are all believed to play a role (47). Patients present with acute renal failure, but the pathology of kidney injury in patients dying of sepsis is of lesser magnitude than is the associated degree of renal dysfunction (48). Tubular injury is typically manifested within the proximal tubules as cytoplasmic volume loss, focal cell detachment and sloughing, cytoplasmic blebbing, and loss of the brush border, findings characteristic of ATN. However, there is no evidence of acute pyelonephritis in most patients with sepsis. Suppurative and emphysematous nephritis are the extreme situations, while most commonly, sepsis is complicated by acute kidney injury (AKI), which can be a serious complication regardless (further discussed in Chapter 24).

Clinical Course, Prognosis, and Therapy of Acute Pyelonephritis

A detailed discussion of the clinical course, prognosis, and therapy of acute pyelonephritis is beyond the scope of this chapter. Outcome data and therapeutic approaches are influenced by the clinical syndromes of "uncomplicated" or "complicated" pyelonephritis. Patients with "complicated" pyelonephritis present with a wide range of structural or functional abnormalities of the urinary tract, with various underlying diseases rendering them more susceptible to infection, or with a renal infection following urologic manipulation. A number of sequelae can complicate the disease including stones, papillary necrosis, pyonephrosis, perinephric abscess, septicemia, and involvement of other organs, for example, the gallbladder (49). Important current issues are what the most common microorganisms causing acute pyelonephritis are and what are the best management and treatment strategies for antibiotic-resistant bacteria in the various age/sex groups (2,50,51).

Chronic Pyelonephritis

Definition and Controversies

The clinical and pathologic features of chronic pyelonephritis appeared over three decades following Löhlein's 1917 article (14) on acute pyelonephritis (52–54). Recognition of a unilateral form of chronic pyelonephritis and its ability to produce hypertension (see Refs. (53,54)) firmly established chronic pyelonephritis as an important disease entity.

Regrettably, during the 1950s and 1960s, many pathologists diagnosed chronic pyelonephritis with such profligacy that it became the most abused term in the whole of renal medicine. The diagnosis was frequently made purely on parenchymal histologic changes, few of which, if any, were specific for the sequelae of infection. The essential "pyelo-" part of the term was completely ignored. It was not until pathologists, including Heptinstall (41,54), took notice of the radiologic observations of Hodson (55) that stricter criteria for the diagnosis of chronic pyelonephritis were imposed. Refinement of our diagnostic criteria has been aided by a better understanding of the mechanisms organisms use to reach the kidney and the pattern of infection produced. These findings in turn have led to problems in nomenclature.

The term *chronic nonobstructive pyelonephritis* gave rise to much confusion in the past. It is understood that vesicoureteral reflux (VUR) and infection may be the initial events leading to parenchymal damage. The possibility that reflux of sterile urine can initiate renal scarring has called into question the term *chronic nonobstructive pyelonephritis*, which by definition implies an infectious origin. Second, it has led to the introduction of the term *reflux nephropathy* (56) to describe the kidney with discrete scars in a lobar distribution. This term displaced "chronic pyelonephritis," specifically the nonobstructive form. Since *reflux nephropathy* does not specify the origin of the scars, it can be used in potential cases of sterile reflux. It is also a more appropriate description of the kidney with severe reflux with generalized pelvic and calyceal dilatation, the so-called *back-pressure* type. The argument against its use is the exclusive emphasis placed on the mechanism whereby urine reaches the kidney, denying the role of infection in scar formation (discussed further later).

Gross Pathology and Light Microscopy

Depending on the site of obstruction, one or both kidneys may be affected. For example, only one kidney will be affected if the obstruction is above the vesicoureteral junction (Fig. 24.15), but both will be involved if the obstruction is below that level. When the renal capsule is stripped, coarse, depressed scars are evident on the cortical surface (Fig. 24.16). The pelvis is

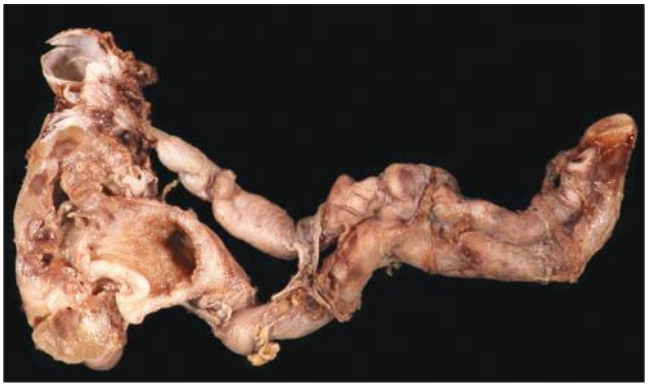


FIGURE 24.15 Chronic pyelonephritis in an 8-year-old girl with left duplicated ureter and multiple recurrent UTIs, but no evidence of renal dysplasia. The kidney is atrophic with thin cortex and dilated pelvis. The contralateral kidney was intact.

dilated as are all the calyceal systems. The pelvic wall is thickened and granular and often shows signs of congestion owing to active infection. Some cases are associated with stones, typically found in the pelvis and calyces. As a consequence of generalized dilatation of the collecting system, it is common to find a thinned parenchyma, particularly in areas aligned with dilated calyces (Fig. 24.17B). Blunting of the papillae is almost invariably a feature. In some cases, large, discrete scars are seen, as in kidneys with the back-pressure type of reflux nephropathy. Often, however, scars are not apparent, and parenchymal thinning is uniform.

Chronic obstructive pyelonephritis shows changes in virtually the entire parenchyma: Tubular atrophy with zones of thyroidization, chronic inflammatory cells, and neutrophils are common in the interstitium, within tubules, and under the pelvic and calyceal mucosa (Fig. 24.17). As inflammation subsides, interstitial fibrosis develops particularly intensely around collecting ducts; a striped pattern of fibrosis—geographic maldistribution—and hyaline degeneration of the ducts of Bellini appear (Fig. 24.18). Lymphoid follicles are present in the parenchyma and under the epithelium of the pelvis and calyces (see Fig. 24.17B). In cases where not all of the parenchyma shows evidence of chronic infection, there may be obstructive atrophy with a paucity of inflammatory cells.

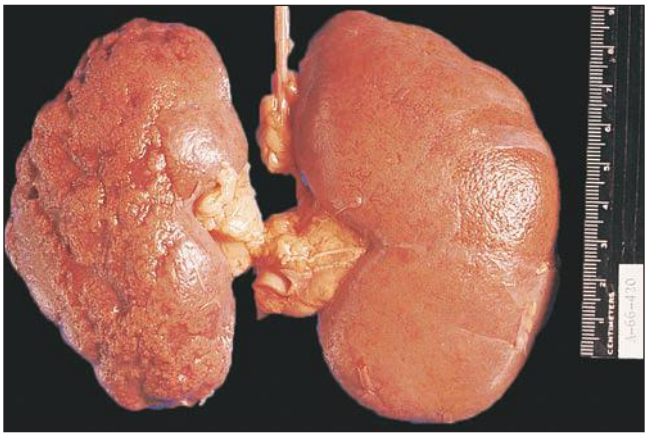


FIGURE 24.16 Chronic pyelonephritis in an adult. Irregular, coarse, depressed scars on the cortical surface of the left kidney are easily appreciated with the capsule stripped.

Specific Forms of Chronic Pyelonephritis

Xanthogranulomatous Pyelonephritis

Clinical Presentation

Xanthogranulomatous pyelonephritis is a rare chronic debilitating inflammatory condition of the kidney characterized by focal or diffuse renal destruction. Xanthogranulomatous pyelonephritis manifests with various combinations of flank pain, a palpable mass, malaise, weight loss, fever, and sometimes nausea and vomiting. There is frequently anemia, leukocytosis, raised erythrocyte sedimentation rate, proteinuria, and white blood cells in the urine. Miscellaneous organisms, including *E. coli*, *Proteus* sp., *Klebsiella* sp., *P. aeruginosa*, and *Enterococcus faecalis*, may be cultured from the urine. In an appreciable number of cases, the urine is sterile (57). Because renal involvement is predominantly unilateral, significant renal failure is rare. Hepatic dysfunction has been reported (58). The reason for hepatic dysfunction is unclear, but abnormal test results often return to normal following removal of the damaged kidney.

Xanthogranulomatous pyelonephritis occurs most often in adult females by a ratio of almost 4:1. The peak incidence is in the fifth and sixth decades. However, it may appear at any age, and patients as young as 2 months (59) and as old as 94 years (60) have been reported. In the pediatric population, boys and girls are affected with equal frequency. In general, it is unilateral. Occasionally, xanthogranulomatous pyelonephritis is bilateral. Usually the entire kidney is involved (generalized form), but restricted forms are not uncommon (57,61).

Enlargement of the kidney, presence of stones or tumors in the renal pelvis, infected areas, and spread of infection to perinephric tissue may all be detected. A high degree of diagnostic precision has been achieved with computed tomography (Fig. 24.19) (2).

Urinary obstruction is an almost invariable feature of xanthogranulomatous pyelonephritis, commonly a result of stones. In large series, stones were found in 78% of cases (57). Stones are frequently of the large staghorn type. Other causes of obstruction include transitional cell tumors of the renal pelvis, congenital pelviureteric stenosis, tumors of the ureter, and postirradiation stricture. In some cases, no cause for the obstruction is apparent (57).

Gross Pathology and Light Microscopy

Grossly, xanthogranulomatous pyelonephritis shares many characteristics with true renal neoplasms in terms of its radiographic appearance and ability to involve adjacent structures (26,60,63). The kidney is enlarged, and adhesions to surrounding renal tissue and perirenal fibrosis are common. In severe cases, the inflammation may spread outside the kidney. Three stages are proposed: Stage I, the lesion is confined to the renal parenchyma; stage II, the lesion involves the perirenal space; and stage III, the lesion extends into perirenal and pararenal spaces (63). The pelvis is dilated and frequently contains staghorn calculi, necrotic material, and pus (Fig. 24.20). Papillae are frequently lost. The parenchyma is brownish and firm from the fibrosis and cellular infiltration. Similar to the

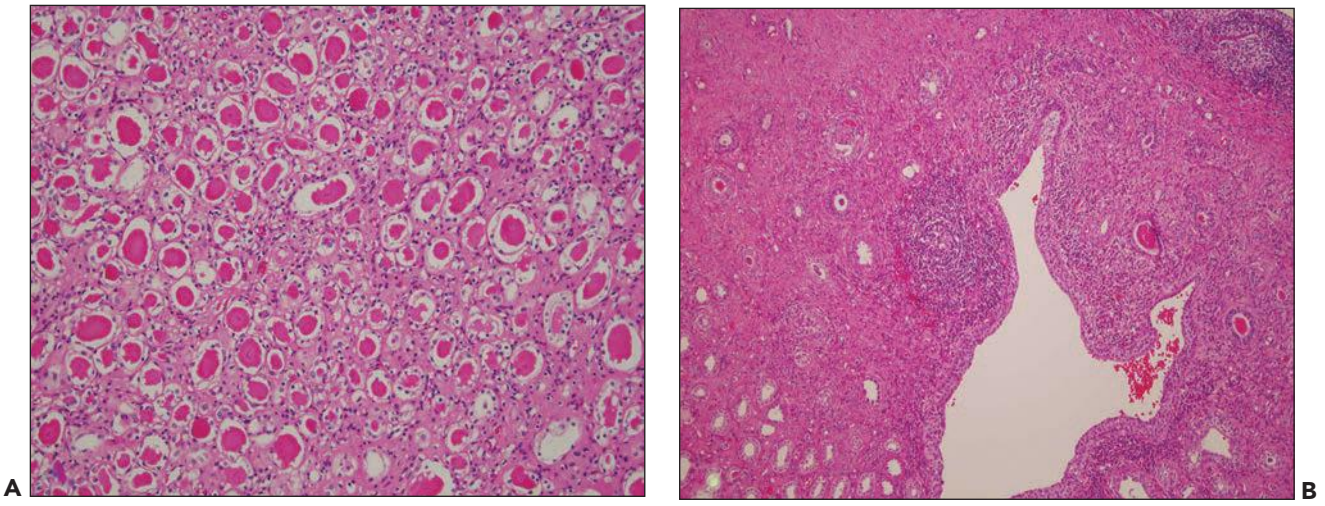


FIGURE 24.17 Chronic pyelonephritis. A: Tubule thyroidization composed of atrophic or dilated tubules with flattened epithelium containing eosinophilic, waxy casts. **B:** The cortex is thin above the dilated calyx. Lymphoid follicles in the cortex and in the pericalicial region, tubular atrophy and pericalicial fibrosis are present. (H&E; ×100.)

calyces, the parenchyma also contains foci of yellowish material. In cases with significant pelvicalyceal dilatation, there may be considerable cortical thinning.

Microscopic examination reveals the yellow areas to consist of large, finely granular foam cells and smaller macrophages containing coarser granules. The large foam cells contain lipid (Fig. 24.21A) and stain positive with CD68. PAS-positive granules may be thinly scattered throughout the foam cells, but they are larger and more prominent in the smaller macrophages. In the outer parts of the yellow zone and the adjacent parenchyma are mononuclear cells, plasma cells, eosinophils, and fibroblasts. Some fibrosis is present in these outer areas as are occasional foreign body giant cells or necrotizing granulomas (Fig. 24.21B and C). Giant cells are found in proximity to cholesterol crystals, which are seen in some cases (see Fig. 24.21A). On the inside of the yellow zone, nearest to the calyx, there is necrotic debris with many neutrophils. Foci of calcification are not uncommon. The overlying cortex shows changes of chronic inflammation and, in some kidneys, microabscesses. Tubular loss is profound. There is interstitial fibrosis and marked interstitial chronic inflammation, composed of lymphocytes, large numbers of plasma cells, and frequent lymphoid follicles. Glomeruli are often normal, but occasionally, may be sclerotic. Thrombosis with recanalization of large veins has been reported in the parenchyma between the yellow areas and the cortex. A pathogenic role for venous obstruction has been suggested (60,63).

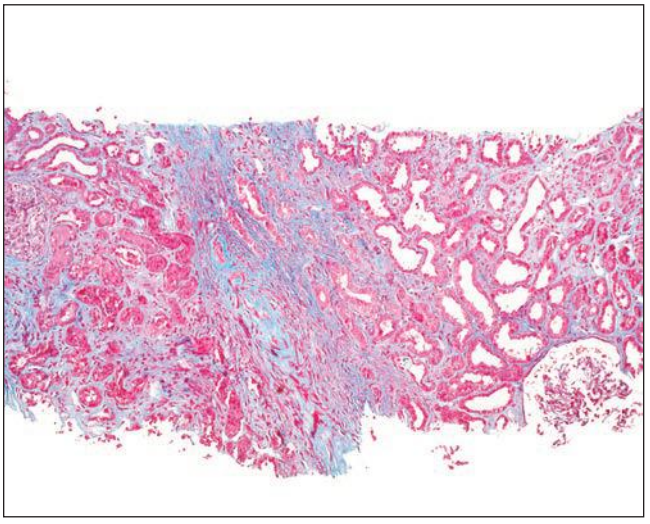


FIGURE 24.18 Chronic pyelonephritis in a 55-year-old man with creatinine of 15 mg/dL secondary to bilateral ureteral obstruction. Renal biopsy was performed to assess extend of damage. Sections show diffuse interstitial fibrosis in a stripped pattern, more pronounced around the collecting ducts. (trichrome stain × 200.)

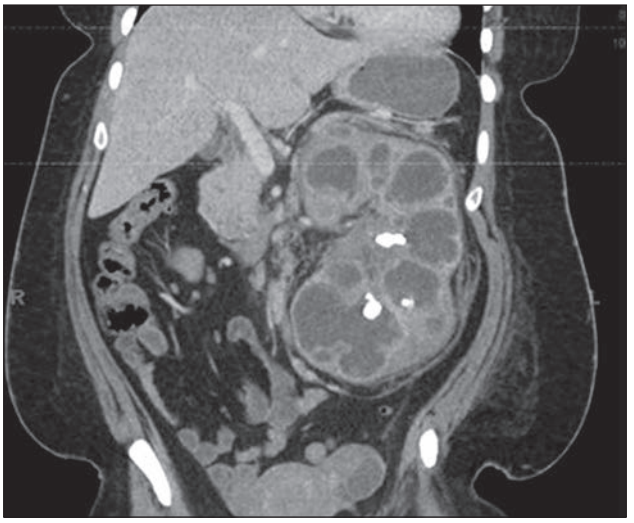


FIGURE 24.19 Xanthogranulomatous pyelonephritis. Contrast-enhanced CT scan demonstrates left kidney enlargement and distension of the collecting system by hypoattenuated material corresponding to inflammatory debris.

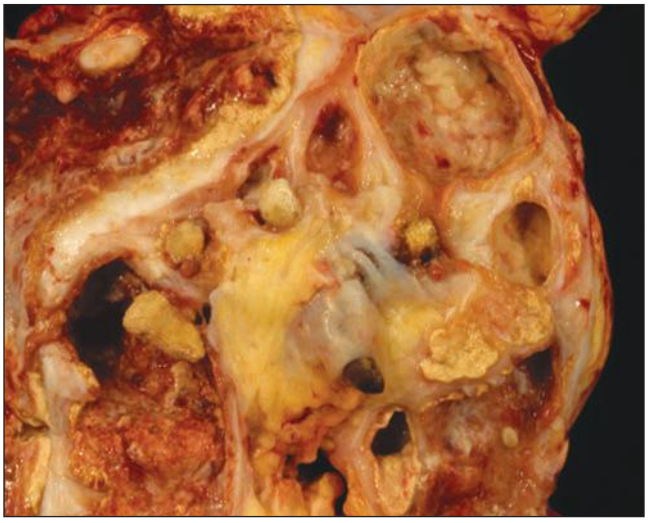


FIGURE 24.20 Xanthogranulomatous pyelonephritis. Friable, yellow tissue surrounds the dilated calyces. Numerous calculi are evident.

The focal variant, which may be mistaken grossly for a clear cell renal cell carcinoma, is restricted to a portion of the kidney with the rest of the kidney appearing normal. The involved areas show pathology identical to the generalized form. Oftentimes, focal xanthogranulomatous pyelonephritis is related to a stone that has formed in a dilated calyx or in cases of duplicated ureter. Segmental resection is curative in the focal form.

Pathogenesis

Xanthogranulomatous pyelonephritis is of infective origin. Its characteristic appearance is likely due to massive parenchymal necrosis and impaired urinary drainage, resulting in accumulation of foam cells. Ultrastructural studies of the lipid-laden macrophages have demonstrated intracellular bacteria within cytoplasmic autophagic vacuoles (64). The histologic appearance may be related to incomplete bacterial degradation and altered host responses.

Malakoplakia

Clinical Presentation

Malakoplakia is an unusual inflammatory condition that occurs in the urinary tract, gastrointestinal tract (mainly the large intestine), testis, prostate, vagina, lung, bone, brain, and skin. Typically, malakoplakia manifests when the immune system is suppressed, for example, in transplant, diabetic, or alcohol-addicted patients. Credit for the first description is given to von Hansemann (65), although two cases had apparently been described earlier by Michaelis and Gutmann (66). Malakoplakia is well known to urologists, who are familiar with the characteristic small, discrete, yellowish-brown plaques or nodules seen on the bladder mucosa during cystoscopy. Similar nodules are found less frequently in the ureter, renal pelvis, and renal parenchyma. When the parenchyma is involved, the nodules are much larger, sometimes involving the entire organ. On renal imaging at various stages, kidneys appear enlarged or nodular mimicking malignancy (2,3,25,26).

A 1993 review by Dobyan et al. indicated females are affected more often than males by a 4:1 ratio for malakoplakia in general and 3:1 for the renal form (67). The age ranges from childhood to the ninth decade, with a mean age for women of 45 years and a peak incidence in the sixth decade for men (67–69). Since the review by Dobyan et al., numerous case reports have been published both in children and adults. Unlike xanthogranulomatous pyelonephritis, which it resembles, malakoplakia often affects both kidneys, causing bilateral disease in about half of the cases.

Clinically, patients present with fever, loin pain, and history of UTI (67). Those with bilateral disease may present with acute renal failure. Signs of perinephric abscess may be noted. The urine contains protein, leukocytes, and sometimes red blood cells. Culture of the urine reveals *E. coli* in most cases. Urinary cytology contributes to the diagnosis by demonstration of characteristic polygonal, granular cells, and Michaelis-Gutmann bodies. Various imaging techniques are employed including CT (2,3). In their review, Dobyan et al. also discussed the role of renal biopsy and pointed out that of the 62 published cases of renal malakoplakia, 18 were accurately diagnosed by either open or needle biopsy. A review by Tam et al. (70) evaluated all cases reported since 1990. The authors emphasized the importance of renal biopsy in establishing the diagnosis. Early identification of malakoplakia allowed for effective therapy using fluoroquinolones, first used to treat malakoplakia in 1990. Renal malakoplakia has traditionally been associated with a substantial mortality rate (70%) and poor recovery of renal function (70). However, the survival rate has increased dramatically to >90% since the introduction of fluoroquinolones (70). Of the 25 cases with long-term

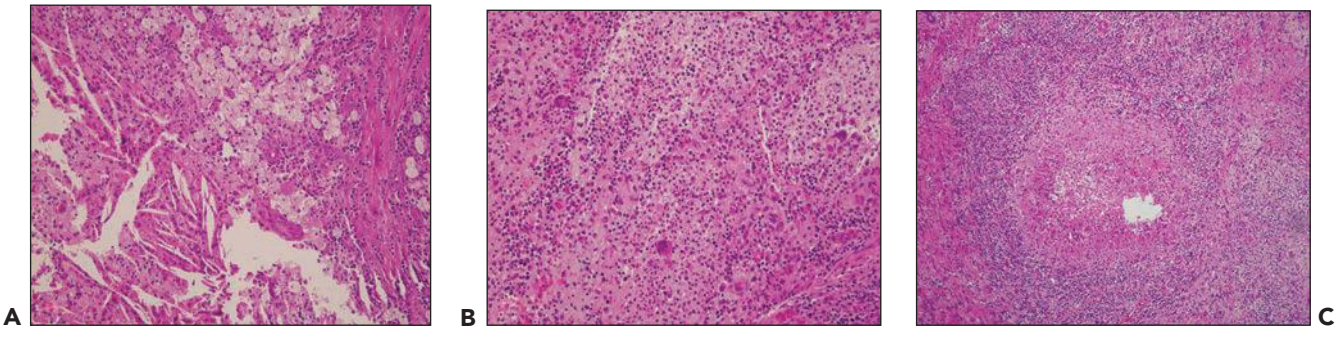


FIGURE 24.21 Xanthogranulomatous pyelonephritis. A: Foamy cells, cholesterol crystals, and granular macrophages. **B:** Giant cells, mononuclear inflammatory cells, and **(C)** necrotizing granulomas are characteristic. (H&E; ×400.)

follow-up analyzed in the review by Tam et al., six developed significant renal impairment with three requiring renal replacement therapy.

Gross Pathology and Light Microscopy

Pathologic changes have been studied at autopsy, in surgically excised kidneys, and in biopsy specimens. On macroscopic examination, the kidney may show effects of obstruction caused with pelvicalyceal dilatation, pus in the pelvic cavity mimicking acute pyelonephritis, and thinning of the renal parenchyma. In contrast to xanthogranulomatous pyelonephritis, calculi are seldom noted in the pelvis and calyces. Perinephric abscesses were documented in 18% of the 62 cases reviewed by Dobyan et al. (67).

Parenchymal involvement consists of yellowish or tan variably sized nodules. These may remain discrete, coalesce to involve much of the renal substance, or undergo suppuration with abscess formation. The nodules are grossly visible on the subcapsular surface. On the cut surface, they can be seen extending down to the papilla. The lesions are sometimes confined to the papilla, which may show necrosis. Yellow areas lining dilated calyces are sometimes evident, but are not as frequent as in cases of xanthogranulomatous pyelonephritis. As is true of the latter condition, malakoplakia may be diffuse or focal. The diffuse variant is much more common.

Microscopic findings in malakoplakia vary and range from lesions mimicking nodular fasciitis, acute pyelonephritis, or fibromatosis. However, classic pathology consists of clusters of moderately large, polygonal cells with a foamy eosinophilic cytoplasm and compact, densely staining nuclei (Fig. 24.22). Within the cytoplasm of these cells are PAS-positive granules and larger inclusions, 4 to 10 μm in diameter, that stain strongly with hematoxylin. These larger inclusions—which may be homogeneous or laminated—are called *Michaelis-Gutmann bodies*. In most instances, they stain with PAS (maintained after treatment with diastase). Prussian blue and von Kossa stains for iron and calcium, respectively, also stain these inclusions. Less consistently, positive staining is obtained with oil red O, alizarin red, and Sudan black B. In addition to the histiocytic infiltrate, mononuclear and plasma cells are also seen. The tubules are severely damaged accompanied by an interstitial fibroblastic and collagenous reaction, which may show a superficial resemblance to a connective tissue neoplasm, ranging from nodular fasciitis to fibromatosis. Unless Michaelis-Gutmann bodies are diligently sought, the diagnosis of malakoplakia may be overlooked. In cases with significant pelvicalyceal dilatation, the unaffected parenchyma may show obstructive changes with features of acute pyelonephritis.

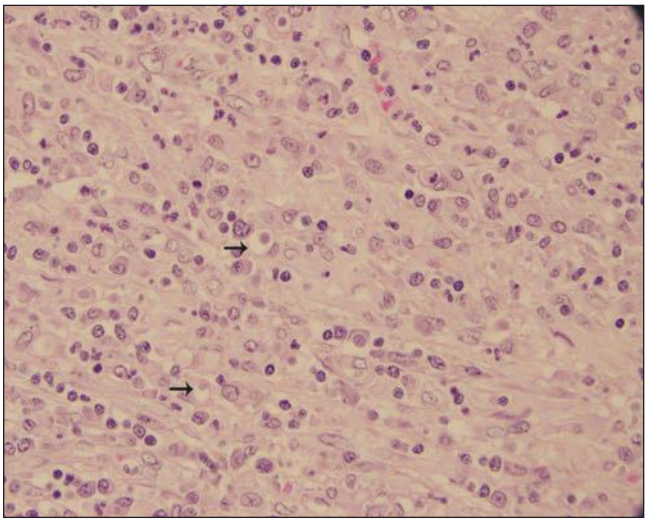


FIGURE 24.22 Malakoplakia. Tubules are destroyed by an interstitial inflammatory infiltrate composed of histiocytes. Numerous intracytoplasmic Michaelis-Gutmann bodies are shown (*arrows*). (H&E; ×200.)

Pathogenesis

The macrophages with PAS-positive granules and the Michaelis-Gutmann bodies have been studied extensively with the electron microscope in both renal and nonrenal cases (71). The PAS-positive granules correspond to phagolysosomes containing complex membranous whorls. The typical picture of the Michaelis-Gutmann body is a central core (often containing crystals), an adjacent lighter zone without crystals, and one or more exterior lamellar rings in which crystals are often found. Complex membranous whorls may be seen at the periphery. These observations led to the belief that Michaelis-Gutmann bodies form by aggregation of crystals upon a nidus of bacterial breakdown products in phagolysosomes. A bacterial origin is supported by studies in rats. Injection of a lipopolysaccharide (LPS) extract of *E. coli* cell walls (Boivin antigen) into kidney or testis reproduces many features of human malakoplakia (72). Injecting low concentrations of Boivin antigen causes macrophage infiltration with a granular cytoplasm. Higher concentrations produced structures similar to Michaelis-Gutmann bodies.

A large number of cases of malakoplakia have been associated with immune system abnormalities or immunosuppressive agents. A role for defective leukocytes and monocytes in the pathogenesis of malakoplakia has received considerable attention. A study of four patients treated with immunosuppressive agents (73) revealed that their leukocytes were unable to kill *S. aureus* and *E. coli*. When the immunosuppressive agents were withdrawn, malakoplakia improved. Abdou et al. (71) found that monocytes derived from a hypogammaglobulinemic patient with widespread malakoplakia had diminished bactericidal activity against *E. coli*. These cells had low levels of cyclic guanosine monophosphate. It was proposed that this resulted in decreased lysosomal degradation and an inability of the cells to release lysosomal enzymes. Cholinergic agonists reversed the impaired bacterial killing activity of this patient's monocytes. Subsequent studies have supported the role of cholinergic agonists in treating malakoplakia (73).

Differential Diagnosis

Several authors have commented on the similarities between malakoplakia and xanthogranulomatous pyelonephritis. It has been speculated that Michaelis-Gutmann body formation is related to the rate at which a nidus is cleared from a focus of infection. Thus, malakoplakia might represent a xanthogranulomatous reaction in which bacterial degradation is abnormal. The same overlap is seen in *megalocytic interstitial nephritis*. The latter is a term applied for a condition characterized by large numbers of polygonal cells with a coarsely granular eosinophilic cytoplasm. The granules stain strongly with

PAS. In contrast to malakoplakia, Michaelis-Gutmann bodies are conspicuously absent (63). Given the striking similarities to malakoplakia, it has been proposed that megalocytic interstitial nephritis is a prediagnostic phase of malakoplakia. The differential diagnosis of all entities above includes malignancy, particularly if the lesion is solitary.

UTI Pathogenesis

Our understanding of UTI pathogenesis has advanced dramatically over the last few decades by discoveries that have shed new light into the complex mechanisms and factors that determine susceptibility to and outcome of infection (74). Pathogenesis involves (a) bacterial virulence, (b) adherence and motility, (c) toxin production, and (d) host-pathogen interactions including evasion of host immune defenses (Table 24.2).

Bacterial Virulence

This special propensity of certain bacteria to cause pyelonephritis, for example, *E. coli*, is due to certain properties of the organisms, collectively known as *virulence factors*. Virulence factors are proteins, toxins, or a structure such as fimbria that enables bacteria to be harmful (74–76). Many novel, putative virulence factors in uropathogenic *E. coli* have been identified using polymerase chain reaction (PCR), including catecholate siderophore receptor (iroN), iron-regulated gene A homologue adhesin (iha), group II capsule (kpsMT), and outer membrane protease T (ompT) (77). It was apparent from early studies that *E. coli* invading the urinary tract had distinct characteristics and only a subset was capable of causing UTI. For example, based on serotyping, the most prevalent *E. coli* serotypes in patients with clinically diagnosed acute pyelonephritis are O1, O2, O4, O6, O7, O8, O16, O16/72, O18, O25, O50, and O75 (78). These strains were found less commonly in cystitis and least commonly in asymptomatic bacteriuria (79). The serotypes were determined by the components of the *E. coli* cell wall (capsule). The wall consists of several layers of polysaccharides such as the O antigen oligosaccharides, K antigens (Kapsel antigen) to distinguish them from the O antigens and the F antigens of fimbriae and flagella. Capsules represent the outermost bacterial layer and impart a mucoid appearance to colonies grown on soft agar. The capsular polysaccharides exhibit extraordinary diversity in structure—dozens are known—but the presence of certain common sugars forms the basis for serologic classification; for example, O, K, and H serotypes are involved in various pathologies. The explanation for the uropathogenicity of the various O serotypes has been the subject of active research that continues to evolve. A new terminology to define virulence is advancing (80). For example, it is now understood that the *E. coli* selection pattern of uropathogenic species is determined by genetic loci known as *pathogenicity islands* (defined blocks of DNA) (81–85). *Escherichia coli* strains 536 (81), IAI, and UMN026 (85) are found to have up to 13 pathogenicity islands (82,83). These sequences represent horizontal gene transfer (HGT)—also known as lateral gene transfer—(86) and can transfer genes from one species of bacteria to another. Gene transfer often involves plasmids and bacteriophages. Such genes include those that confer antibiotic resistance; therefore, HGT plays an important role in the maintenance and transmission of virulence and is the primary reason for bacterial antibiotic resistance (86).

A putative uropathogenic island including a gene encoding uropathogenic-specific protein (USP) has been identified in *E. coli* strains isolated from patients with pyelonephritis (77).

Adherence and Motility: the Role of Fimbriae

Much attention has been given to the adhesion of bacteria to mucous membranes to explain colonization of the urinary tract. Adhesins include fimbriae, adherence pedestals (similar to fimbriae), and afimbrial adhesins, such as polymers, polysaccharides, lipoteichoic acid, and high molecular weight proteins. Whereas Gram-positive bacteria adhere more frequently via extracellular polysaccharides, Gram-negative bacteria utilize fimbriae (Fig. 24.23). Adhesion is affected by interactions between epithelial cell surface receptors and hair-like appendages termed fimbriae or pili found on the surface of the infecting organism. Fimbriae are broadly divided into two main groups, depending on the ability of mannose to interfere with their ability to attach to receptors. Thus, they are designated *mannose sensitive* or *mannose resistant*. Type 1 fimbriae are mannose sensitive, while P fimbriae and X fimbriae are mannose resistant. P fimbriae (so called because they attach to a digalactoside residue [Gal–Gal] related to P blood group antigens on human erythrocytes and uroepithelial cells) appear to be most important in

TABLE 24.2 UTI pathogenesis: key facts

Bacterial factors	Host factors
Bacterial virulence	Antibody production against bacteria
Adhesion	pH (vaginal flora)
Motility	Tamm-Horsfall protein (THP)
Toxin production	CXCR1 gene polymorphisms
Intracellular communities (IBCs)	Neutrophils
Quiescent intracellular reservoirs (QIRs)	Innate immune response—IL, TLR

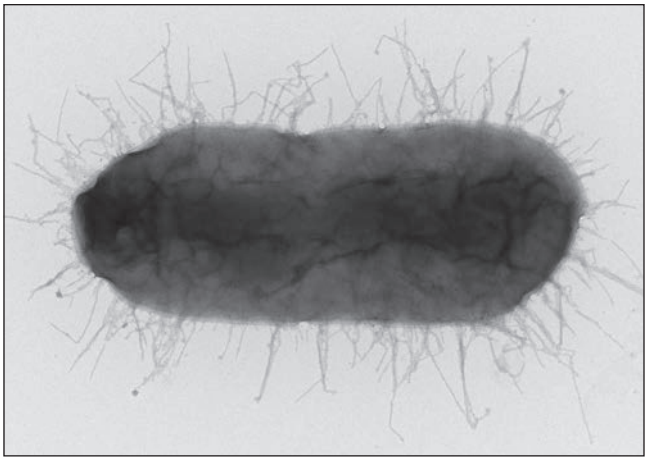


FIGURE 24.23 Uropathogenic *E. coli* bind to urinary epithelium with fimbriae (pili). P fimbriae (for pyelonephritis-associated pili) are important virulent factors; only bacteria featuring P pili cause pyelonephritis. (electron microscopy–negative stain × 20,000). (Courtesy of Scott Hultgren, Washington University School of Medicine, St. Louis, MO.)

UTIs, especially with regard to renal involvement. The receptor-binding adhesin at the tip of P fimbriae is pap G. There are three classes of G-tip proteins (87). Class II–tip adhesin is associated with pyelonephritis and class III–tip adhesin with cystitis.

Fimbriae of uropathogenic *E. coli* contain distinct gene clusters known as operons that code for proper assembly of the structural components of fimbriae and other proteins that control their function. For example, uropathogenic *E. coli* strain CFT073 has 13 fimbriae gene clusters including type 1, P, F1C, Dr, Auf, S, and M. P fimbriae are associated with human pyelonephritis but cause no disease in mouse models of UTI (88). Dr harboring *E. coli* adhere to bladder epithelium and enable cell invasion in vitro, in contrast to Auf fimbriae that play no role in colonization of the urinary tract in experimental animals. The genetically and chemically distinct forms of fimbriae are under the control of a promoter upstream of the genes of interest. When the promoter is on (in the correct orientation in the gene sequence), fimbriae form; when it is off (in the opposite direction), production of fimbriae is halted. The promoter is supervised by multiple enzymes, for example, Fim B and Fim E (89), and affected by local factors such as pH, oxygen supply, and presence of antibodies.

Motility is mediated by bacterial surface structures called flagella. *Escherichia coli* use flagella to ascend from the lower to upper urinary tract in a process that appears highly regulate. For example, *E. coli* flagellae are present in ascending infection and are decreased in chronic infection (90). *Proteus mirabilis* has a swarming mobility that transforms this bacterium to an elongated form enabling it to move across the surface of urinary catheters and cause invasive UTI (91,92).

Toxin Production

Escherichia coli produce three types of toxins: hemolysin, cytotoxic necrotizing factor 1 (CNF1), and autotransporter secreted toxins. Hemolysin toxins enable bacteria to insert into host cells including urothelial cells. Invasion triggers cytokine production leading to inflammatory response (93). CNF1 toxin is also implicated in invasion of host cells by altering their cytoskeleton and causing exfoliation of bladder epithelial cells, thus exposing submucosal tissue to bacteria (94). CNF1-positive *E. coli* stains cause more inflammation compared to bacteria lacking CNF1 (95).

Host-Pathogen Interactions

Antibodies Against Bacteria

There is experimental evidence that antibodies can protect against bacteria invading the urinary tract. Their role in humans is more difficult to assess. Antibodies may be produced either locally or systemically (96,97). Experimental studies support the claim that finding antibody-coated bacteria in the urine in human patients indicates kidney infection, as opposed to lower UTI (98).

In human acute pyelonephritis caused by *E. coli*, antibodies are produced against O and K antigens (96) and fimbriae (99), principally as a systemic response. IgM is dominant in the early stages, followed by IgG and IgA. The urine of patients with pyelonephritis also contains IgG and IgA. IgA is probably produced by local mechanisms (17). The efficacy of these antibodies in humans is difficult to ascertain, but their presence explains the fact that recurrent UTIs are usually caused by different strains, the original strains having been eradicated by antibodies.

Vaginal Flora

Various mechanisms operate to prevent attachment of uropathogens to epithelium of the vagina, periurethral region, and urethra. Endogenous bacteria, such as lactobacilli in the vagina, protect by lowering local pH and by interfering with attachment via steric hindrance, competition for receptor sites, and inhibition of bacterial growth (100). More recently, using molecular-based techniques, healthy vaginal microflora was found to lack high numbers of many "good" *Lactobacilli* species. Instead, one or two lactobacilli from a range of three or four species are dominant, whereas other species are rare (101). It appears that the disease known as bacterial vaginosis is due to different bacterial profiles of greater microbial diversity than is evident from cultivation-dependent studies. These studies hold promise to solve the mystery of why a good number of women are prone to UTIs.

Host Defense Mechanisms

Antiadherence mechanisms abound in the bladder. First, there is a layer of urothelial glycosaminoglycans (102), which prevents the attachment of pathogens. Second, there is the so-called slime or uromucoid layer (now recognized to be Tamm-Horsfall protein [THP]), which lines the bladder, shows great avidity for *E. coli* bearing type 1 fimbriae, and thereby protects the underlying mucosa (103). Experimental infection in mice supports an antimicrobial, protective role for THP, and in addition, it appears that THP also exerts immunoregulatory activity (103,104). THP knockout mice develop severe infection and lethal pyelonephritis in an experimental model of UTI (104). Furthermore, recent data demonstrate that THP links the innate immune response with specific THP-directed cell-mediated immunity via IL-1β release from inflammatory cells (monocytes) (105). Immunoglobulins in the urine of patients with acute pyelonephritis are capable of reducing adherence of *E. coli* to urothelial cells (17). Urine supports the growth of bacteria, but because of its relatively low pH, high osmolality, and high urea content, it is not the ideal culture medium (106). When glucose is present in the urine, as in diabetes mellitus, conditions are more propitious for bacterial growth. Voiding is also an important factor in preventing infection. Its importance becomes apparent when it is impaired, such as in obstruction of urinary outflow and VUR.

Persistent *E. coli* infection induces mucosal cells to produce interleukin-6 (IL-6) and IL-8, which initiate a local inflammatory response (100). Neutrophils abound in the acute phase and are seen adjacent to bacteria (Fig. 24.24); they kill bacteria by phagocytosis and release of intracellular proteolytic enzymes and reactive oxygen species. Also, neutrophils degranulate, releasing bactericidal enzymes extracellularly. Extracellular killing of bacteria by neutrophils is achieved with extracellular fibers that capture bacteria (107). The fibers are called neutrophil extracellular traps (NETs) and are only made by activated neutrophils. NETs contain neutrophil elastase, cathepsin G, and myeloperoxidase (MPO) and other enzymes derived from neutrophil granules. NETs target both gram-positive and gram-negative bacteria. It appears that the purpose of bacterial NETs is to prevent phagocytosis (107). While the aim is achieved efficiently, tissue damage ensues in part because of the toxic effects of granular enzymes to the tissue but also because NETs contain DNA including histone particles acting as immune modulators causing chronic damage similar to autoimmune diseases (40,107).

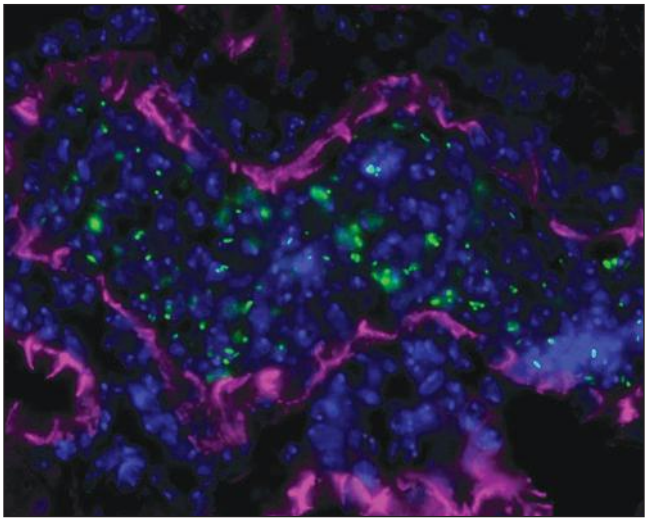


FIGURE 24.24 Experimental pyelonephritis: uropathogenic *E. coli* (green FITC) induce influx of neutrophils (Hoechst nuclear blue) in tubules (labeled pink with E-cadherin). Mouse kidney immunofluorescence × 63. (Courtesy of Indira Mysorekar, Washington University School of Medicine, St. Louis, MO.)

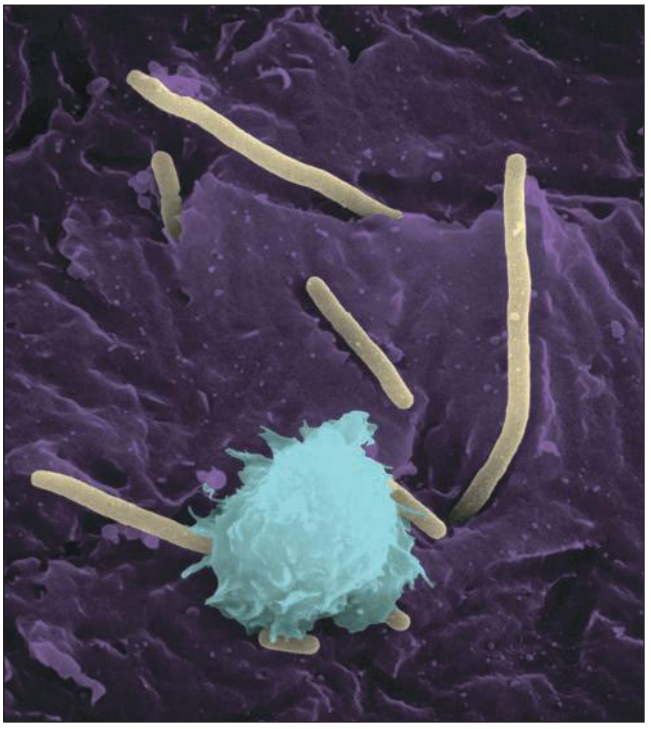


FIGURE 24.25 Scanning EM of urothelial mucosa with adherent bacteria reveals uropathogenic *E. coli* adhering to and colonizing the urinary bladder (magenta). Inflammatory cells (light blue) are recruited to contain bacterial invasion (pale yellow). (Courtesy of Chia Hung and Scott Hultgren, Washington University School of Medicine, St. Louis, MO.)

Susceptibility Factors

Blood group antigens and secretor status were found to correlate with increased susceptibility to UTI. Secretors differ from nonsecretors in their ability to secrete water-soluble blood group antigens. An increased risk of recurrent UTIs was found among women of blood groups B and AB who were nonsecretors (108). It has also been shown that women who were nonsecretors were unduly prone to renal scarring following recurrent UTIs (109). Uroepithelial cells from nonsecretors adhere with greater avidity to uropathogenic *E. coli* than do cells from secretors (110). Recent studies have focused on adhesive properties of bladder and kidney epithelial cells to evaluate how interactions between bacteria and host epithelia may facilitate bacterial adhesion and colonization (Fig. 24.25). For example, studies have shown that urothelial cells express a hyaluronic acid–binding protein, adhesion molecule CD44, which is constitutively expressed in the bladder and acts as a receptor for adhesion of bacteria such as *Streptococcus*. In the urinary bladder, it facilitates *E. coli* adhesion and accumulation. CD44 is not expressed on tubular epithelial cells. However, following tubular injury, CD44 is rapidly up-regulated at the tubular epithelial cell surface facilitating *E. coli* migration (111). Disruption of CD44 and hyaluronic acid in the bladder dramatically decreased bacterial overgrowth in spite of similar granulocyte and cytokine response in a murine UTI model, suggesting that this may be another approach to prevent and/or treat UTI (111).

The innate immune response of the host is important in the antibacterial defense mechanisms of the urinary tract, and normally, bacterial clearance proceeds without sequelae. Innate immune response is triggered by bacteria-induced influx of inflammatory cells (neutrophils and monocytes) and damage of epithelial cells that express toll-like receptors (TLRs). About 13 TLRs are identified; the first to be identified was TLR4, which recognizes LPSs from gram-negative bacteria (112,113).

A variety of host genetic susceptibility factors have been identified within the innate immune response molecules that are associated with increased incidence of acute pyelonephritis, for example, interferon regulatory factors (IRFs) and type 1 interferons. A strong association between decreased IRF3 promoter activity and acute pyelonephritis in humans was demonstrated. IRF3 is a gene encoding the IRF-3 protein. This protein is part of the TLR4 signaling pathway. Knockdown of Irf3 in mice impaired neutrophil bactericidal activity and resulted in severe disease with urosepsis and abscess formation (114). Other studies suggest a role for the CXCR1 gene, encoding the chemokine receptor type 1 protein, in acute pyelonephritis susceptibility. CXCR1 is important for neutrophil recruitment to sites of infection. Diminished expression of this protein was found in children prone to developing acute pyelonephritis (115,116). Single nucleotide polymorphisms in other genes involved in the innate immune response including CCL5, TLR4, VEGF, and CXCL8 were also found in association with human acute pyelonephritis (reviewed in Refs. (116,117)).

Bacteria and Host Immune Defenses

Breaking through mucosal barriers and immune defenses, bacteria employ ingenious approaches to succeed in establishing infection in the urinary tract. For example, *E. coli* expressing P fimbriae are shown to down-regulate the polymeric Ig receptor (pIgR) produced by renal epithelial cells, which transports IgA in the urinary tract (118). Decrease of pIgR correlates with IgA decrease in P fimbriae–positive *E. coli*–induced UTI in mice (118).

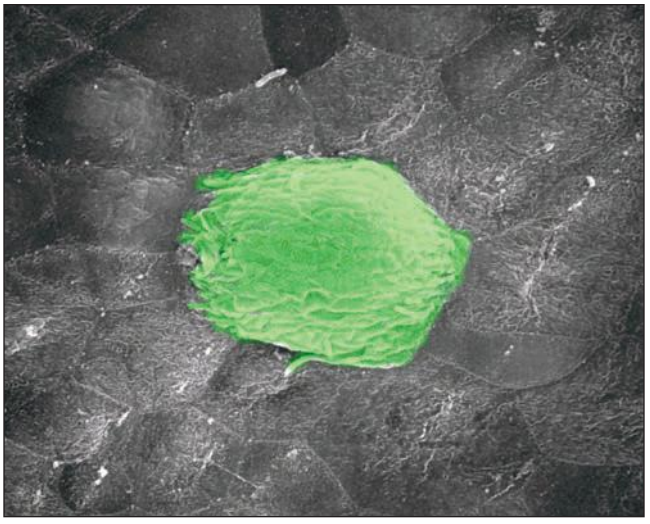


FIGURE 24.26 Scanning EM of an intracellular bacterial community (IBC) on the urothelial surface. *Escherichia coli* shelter from host defenses leading to persistent bacterial residence within the bladder epithelium. IBCs never form in the kidney; only form in the urinary bladder. Exfoliation of the superficial urothelial layer acts to reduce the bacterial load but facilitates chronic residence of small nests of bacteria that later reemerge to cause recurrent cystitis. (Courtesy of Indira Mysorekar.)

Further, uropathogenic *E. coli* evade immune response via intracellular bacterial communities (IBCs) (Fig. 24.26) and quiescent intracellular reservoirs (QIRs). IBCs are intracellular biofilms defined as assumption of a temporary multicellular lifestyle by a single-cell organism in which "group behavior" facilitates survival in adverse environments. Under certain conditions, IBCs are protected from host immune response such as neutrophil influx and antibiotics, in spite of shedding of epithelial cells triggered by the infection. It appears that inside the host cells, *E. coli* manage to subvert immune responses in part through autophagy, a physiologic self-cleaning mechanism; epithelial cells use autophagy to degrade toxic cytoplasmic substances, but microorganisms can utilize this mechanism to their advantage (119). Bacteria eventually exit their intracellular reservoirs seeding new colonies leading to persistent or recurrent infection.

Risk Factors

Obstruction to Urinary Outflow

Obstruction is a potent factor not only in initiating infection but also in causing it to persist and spread to the kidney. Obstruction below the bladder neck results in loss of the "flushing mechanism," which, together with incomplete emptying of the bladder, permits bacterial growth in relatively static residual urine. The use of catheters is an additional hazard for introducing bacteria and for permitting bacterial growth (120). Urine flow obstruction complicates various cystic diseases as well, autosomal dominant polycystic kidney disease in particular, further discussed in Chapter 4.

Diabetes Mellitus

Diabetes mellitus is cited as a risk factor for UTIs and their side effects (121). Claims for certain acute infections of the upper urinary tract stand on solid foundations. For example, patients with diabetes are described as being more prone to cortical abscesses, perirenal abscesses, and emphysematous pyelonephritis (2,3). There is little evidence of an increased prevalence of true chronic pyelonephritis in the diabetic population.

Calculi

Calculi affect the host's defense mechanisms in several ways. First, they cause obstruction, which may occur at various levels. Second, they may serve as a nidus for the persistence of infection, either because they act as an irritant or because they harbor organisms, making them difficult to eradicate (122). Calculi may aggravate infections and, in the case of struvite stones, be caused by infections. Struvite stones are composed of magnesium ammonium phosphate caused by bacterial urease activity, for example, in the presence of urea-splitting bacteria such as *Proteus* sp.

OTHER INFECTIONS

Renal infections are a major cause of morbidity and mortality particularly in immunocompromised patients such as AIDS and transplant recipients (123).

Mycobacterial Infections

After continued decline for three decades, pulmonary tuberculosis is again increasing. It is the leading cause of death worldwide from infectious disease (124). In Western countries, the prevalence of renal involvement among patients with tuberculosis is around 5% (125). In African countries, the prevalence of renal tuberculosis is probably higher. In Nigeria, the prevalence of renal tuberculosis among patients with pulmonary tuberculosis was found to be 9.5% if diagnosed using urine Ziehl-Neelsen stains, but the prevalence rate rose to 14% if a combination of urine stains, sterile pyuria, and tissue histology was used (124). Therefore, the diagnostic methods clearly influence the prevalence rate. Newer, more sensitive methods, including PCR, may improve diagnostic accuracy.

Immunosuppressed patients are more vulnerable to mycobacterial infections. Transplantation is a risk factor, particularly in developing countries (126). The human immunodeficiency virus (HIV) epidemic has had a significant impact on the spread of tuberculosis worldwide, particularly in Africa (123,124). Approximately 10% of tuberculosis cases worldwide were HIV related, but in regions of sub-Saharan Africa, the percentage was as high as 60%. There are two main types of renal tuberculosis: miliary and cavitary.

Miliary Tuberculosis of the Kidney (Disseminated Infection)

Renal involvement may result from hematogenous dissemination of a primary tuberculous infection, from an active pulmonary lesion, or from reactivation of a healed tuberculous lesion. Miliary tuberculosis of the kidneys is often clinically silent (27,127) and overshadowed by clinical manifestations of systemic infection. Grossly, the kidneys show white nodules (i.e., tubercles), which occur more often in the cortex than in the medulla, but they may occupy the entire kidney (Fig. 24.27). Microscopically, the early tubercle is a caseating granuloma consisting of epithelioid cells and neutrophils with central

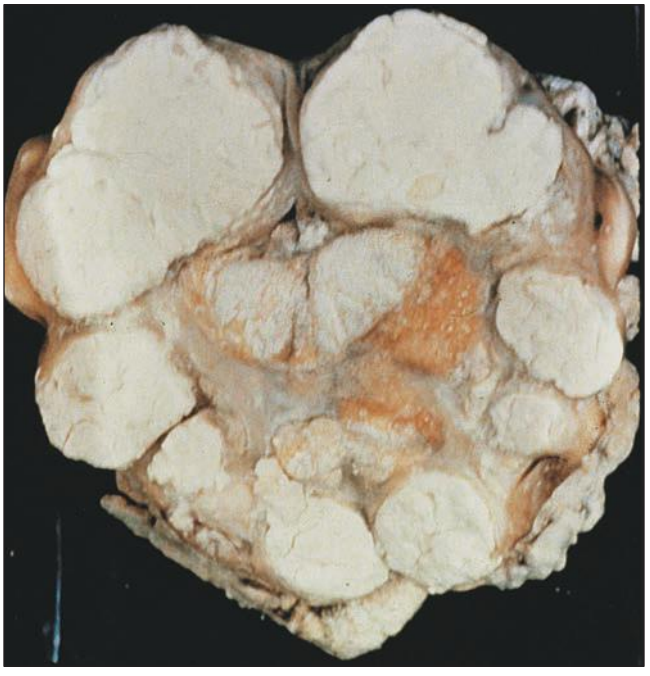


FIGURE 24.27 Tuberculous pyonephrosis. Kidney is filled with cheesy material.

caseous necrosis (Fig. 24.28). Organisms are usually found in such lesions. Often, a mononuclear infiltrate of lymphocytes, monocytes, and plasma cells is also present. The tubercle may be contained and heal, or the infection may expand. If the medulla is involved, the infection may reach the renal pelvis, allowing release of microorganisms into the urinary tract.

Cavitary Tuberculosis of the Kidney (Localized Urinary Tract Infection)

A high proportion of men with renal tuberculosis have associated genital tuberculosis, particularly affecting the epididymis and, less frequently, the prostate (123). Genital tuberculosis is less common in women (if present, it is usually in the fallopian tubes). The lower urinary tract is commonly affected in cavitary renal tuberculosis raising the possibility of ascending infection. However, it is believed that descending spread to the urinary tract from a primary renal lesion is more likely. The renal medulla is preferentially involved by cavitary tuberculosis, where confluent epithelioid caseating granulomas will form larger and larger cavities, frequently associated with papillary necrosis (27). In cavitary renal tuberculosis, also called caseous and ulcerative, most of the symptoms result from lower urinary tract involvement, particularly the urinary bladder. The disease manifests with urinary frequency, dysuria, and hematuria. Commonly, sterile pyuria and microscopic hematuria are present. Renal function is typically preserved since unilateral renal involvement is common. Usually presents as a unilateral disease, but the contralateral kidney is involved to some degree (26,27). The diagnosis of renal tuberculosis requires a positive culture for mycobacteria, rhodamine-auramine fluorescence, or urine PCR for mycobacteria.

Gross pathology of the kidneys shows either enlarged or decreased size. The surface shows irregular scarring. On cut section, the calyces and the pelvis are dilated or deformed, pelvicureteric constriction may occur, and parenchymal atrophy and foci of calcification are apparent. The lesion often begins in the renal medulla with involvement of the papilla by caseating necrosis (27). Extension of infection into the perinephric tissues may simulate invasive renal cell carcinoma (3). Segmental ureteral strictures may be seen with ureteral involvement. A combination of pelvicalyceal caseous necrosis and ureteral stenosis leads to tuberculous pyonephrosis. When this occurs, the renal parenchyma is replaced by caseous material, leaving rims of fibrous tissue imparting a loculated appearance to the organ. This condition is also known as "cement," "putty," or "chalk" kidney (see Fig. 24.27).

Microscopically, these lesions show central caseous debris surrounded by a peripheral granulomatous reaction (see Fig. 24.28). Mycobacteria are found in peripheral areas of caseation or cavitary lesions. Less involved areas may show

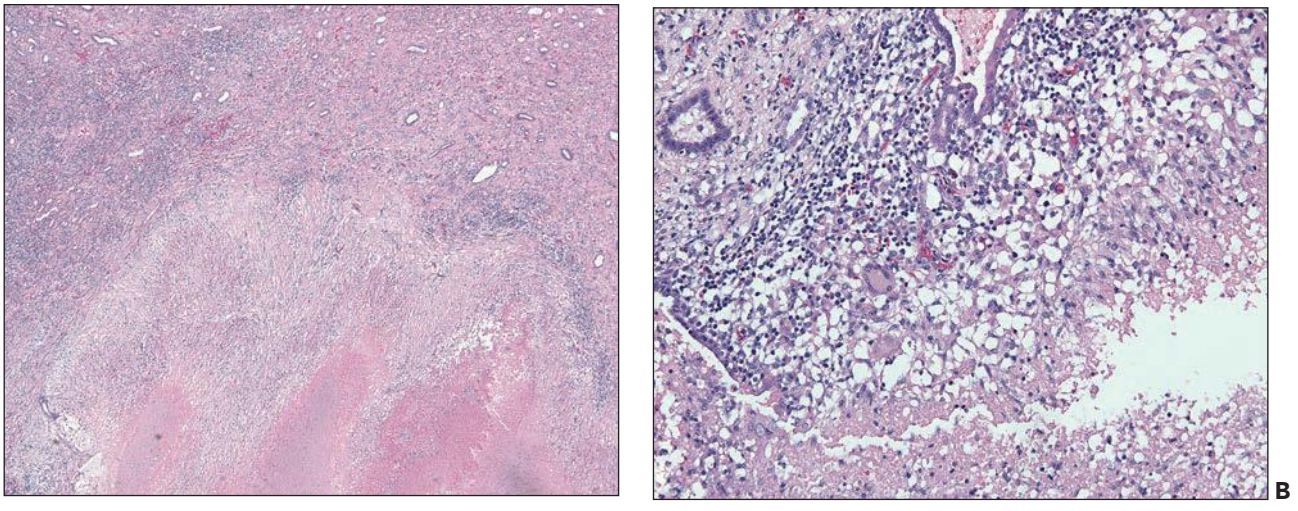


FIGURE 24.28 A: Caseating granulomas with central necrosis and epithelioid histiocytes at the periphery. **B:** Giant cells are appreciated. (H&E; **A**, ×100; **B**, ×400.) (Courtesy of Neeraja Kambham, Stanford University, California.)

variable interstitial inflammation with lymphocytes and plasma cells amidst calcific foci, probably representing calcified tubercles.

In renal biopsies, tuberculosis is rarely seen in developed countries (128). Interstitial nephritis and epithelioid or caseating granulomas may be present, but stains for acid-fast bacilli are usually negative. Findings of acute interstitial and granulomatous nephritis in patients with documented extrarenal tuberculosis can be the result of drug reaction, and kidney biopsy should be performed to rule out active renal involvement or changing therapy. However, it is imperative to request a mycobacterial stain (acid-fast blue or Ziehl-Neelsen) if granulomas are present in the biopsy specimen, particularly if the patient's immune system is compromised. Also, it is important to remember that in immunocompromised patients, atypical mycobacterial infections can occur, including infections with *M. avium-intracellulare*, which does not form typical granulomas. In such cases, abundant mycobacteria are present in foamy-appearing macrophages.

Mycobacterium leprae

Visceral involvement is more common in the lepromatous leprosy than in tuberculoid leprosy (129). Tuberculous (paucibacillary) leprosy is characterized by epithelioid granulomatous inflammation, whereas lepromatous leprosy (multibacillary) has a tendency toward diffuse infiltration of macrophages and foamy bacilli-laden cells. Renal injury is common in patients with erythema nodosum leprosum, a type of reaction typically associated with lepromatous leprosy (129).

Renal lesions are found in approximately 70% of autopsied patients with leprosy. The glomeruli, tubules, and interstitium may be involved causing glomerulonephritis, including amyloidosis and acute interstitial nephritis (130,131). Histologic examination may show endocapillary proliferation, mesangial proliferation, membranoproliferative glomerulonephritis (MPGN), and, less commonly, crescentic glomerulonephritis. In addition to glomerular pathology, chronic tubulointerstitial nephritis has also been observed in 3.8% to 54% of cases (131). Organisms are not detected in the kidney in such cases. The inflammatory infiltrate is mononuclear with associated interstitial fibrosis and tubular atrophy. Renal infection appears to occur in patients who have undergone prolonged chemotherapy for leprosy. Leproma caused by direct invasion of the renal parenchyma by *M. leprae* is rare. When present, bacteria can be identified in aggregations of macrophages or giant cells.

Pathogenesis

Granulomas are the hallmark of mycobacterial infection, but fungi, parasites, and even viruses cause granulomatous inflammation. The mycobacterial granuloma seems to be a host defense mechanism for walling off the bacilli, but microorganisms can survive within macrophages that compose the granulomas and persist in a latent form until immunosuppression and other triggers cause reactivation and dissemination. An understanding of the pathophysiology of granulomas is critical for the design of new drugs and vaccines. Animal models including mice, guinea pigs, rabbits, and monkeys, and in vitro systems were developed that reproduce granulomatous inflammation (132). In addition, the role of innate immunity was explored asking the question how mycobacteria manage to escape killing by immune cells. Master et al. (133) showed that *Mycobacterium tuberculosis* prevents inflammasome activation and IL-1β processing and that a functional *M. tuberculosis* zmp1 gene is required for this process. Experimentally induced infection of macrophages caused increased secretion of IL-1β and enhanced mycobacterial phagosome maturation into phagolysosomes, thus improving mycobacterial clearance by macrophages (40,114,133).

Fungal Infections

Primary fungal infections of the kidney are rare. The most common offending agents are *Candida albicans* and *Candida glabrata*. Such agents infect the lower urinary tract and may involve the kidneys through the ascending route. However, fungal infections of the kidney more commonly result from systemic fungemia, possibly of nosocomial origin and almost invariably occur in immunocompromised patients, particularly those undergoing cytostatic treatment for malignancies. In such cases, renal involvement is part of systemic fungal sepsis. Irrespective of the type of fungus infecting the kidney, the renal biopsy typically shows granulomatous inflammation. Therefore, when granulomatous inflammation is seen, a fungal infection should be considered in the differential diagnosis. Importantly, the PAS stain, excellent for detecting fungi, should be part of routine renal biopsy workup. Some fungi, for example, Aspergillus, tend to be angiocentric, as opposed to Candida, which tends to be glomerulocentric.

Candidiasis

Most infections by *C. albicans* are opportunistic. Infections originate from mucosal surfaces of the oral cavity, upper respiratory tract, digestive tract, and vagina, followed by hematogenous dissemination. The source of infection often is nosocomial (134). Candidiasis typically affects patients with prolonged hospitalization, with 50% of Candida bloodstream infections occurring in the ICU (134). The mean time of onset of systemic Candida infections is 22 days after hospitalization. Furthermore, when skin/mucosal barriers are breached by medical devices or surgery, it opens a portal of entry for pathogens like *C. albicans*. For instance, major abdominal surgery poses an increased risk for systemic Candida infections, which is underlined by the observation that in a cohort of 107 patients with candidemia, 50% underwent recent surgery (134).

The incidence of candidiasis varies. Of 102 episodes of nosocomial fungemia, *C. albicans* was detected in 74.5% (134). Presenting symptoms are those of severe renal infection, with low-grade fever, flank pain, costovertebral angle tenderness, hematuria, hypotension, progressive loss of renal function, and acute renal failure. Fungus balls may develop in the pelvis and calyces (135), and their passage may result in ureteral colic. Anuria occurs when fungus balls obstruct the ureters (135). Recovery of *Candida* from urine specimens, together with positive blood culture, suggests disseminated infection.

The kidneys may show little inflammatory response, or there may be extensive necrosis, or miliary abscesses (Fig. 24.29), and papillary necrosis. In those with diabetes, *C. albicans* infection may result in emphysematous pyelonephritis. Mycotic aneurysms may be present in glomerular capillaries and arterioles. However, in comparison with other fungi, invasion of blood vessels by *Candida* is less common, and cortical infarction

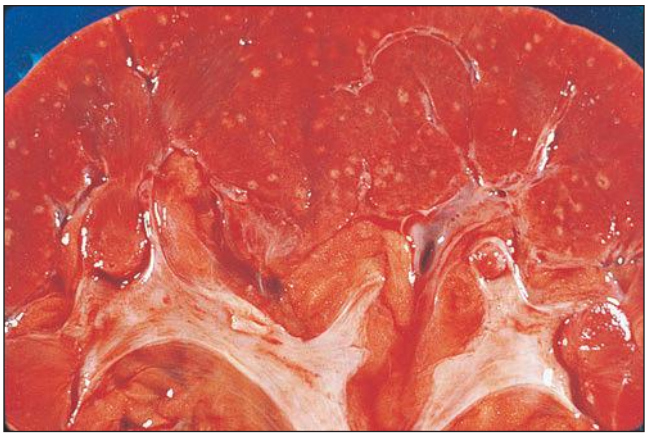


FIGURE 24.29 *Candida* septicopyemia with scattered small, yellow-white miliary abscesses.

is rare (136). In tissue sections, pseudohyphae and rounded yeast forms, 2 to 4 μm in diameter, predominate (Fig. 24.30). Although *Candida* can be seen in sections stained with hematoxylin and eosin, they are more readily identified with PAS or Grocott methenamine silver stains.

To colonize surface epithelia, *Candida* organisms adhere to epithelial cells through mannoproteins, hydrophobic forces (137), and proteins that bind iC3b receptors. The *C. albicans* cell wall can be divided into two distinct layers: the inner layer composed of polysaccharides like chitin, 1,3-b-glucans and 1,6-b-glucans, and the outer layer composed of proteins that are heavily mannosylated. These Candida molecules are recognized by Toll-like receptors (TLRs) and C-type lectins (CLRs) on the surface of antigen-presenting cells (APCs) (138). Unless phagocytosed, *Candida* organisms reach the subepithelial layer where through surface receptors bind to extracellular matrix components. During colonization and penetration, epithelial proliferation and T-cell–based inflammation are elicited. Animal models have shown that *C. albicans* can escape killing by mouse neutrophils but is efficiently cleared in human neutrophils probably due to higher levels of MPO activity and the presence of α-defensins (139). Only a minority of patients exposed to Candida develop disease. These include patients with defective T-cell function or immunosuppression who are unable to adequately contain the fungus. As a result, invasion of the vascular endothelium ensues. More recently, susceptibility to Candida was found to be linked to defective innate immunity either due to single gene mutations (rare) or due to common polymorphisms in pathogen recognition receptors such as TLRs (138).

Candida glabrata

Candida glabrata (*previously classified as Torulopsis glabrata*) (140) is an opportunistic yeast-like fungus present in the normal microflora of the oropharynx, gastrointestinal tract, skin, urethra, and vagina. Of low virulence, *C. glabrata* is the second most common fungal pathogen of the urinary tract. The kidneys are usually involved as part of disseminated infections, but they may be the site of a primary infection through the ascending route, particularly in diabetic patients. *Candida glabrata* is also an important cause of nosocomial infection. Presenting symptoms are comparable to those caused by infection with *C. albicans.* Inflammatory changes resemble those caused by *Candida albicans* infection. *Candida glabrata* organisms may be seen in tissue sections as 2- to 4-μm, budding, round to oval, nonencapsulated, yeast-like organisms (Fig. 24.31).

Aspergillosis

Aspergillosis can be caused by various species of aspergilli, but the most common pathogen is *A. fumigatus*. The organism, present in nature, typically enters the host through respiratory

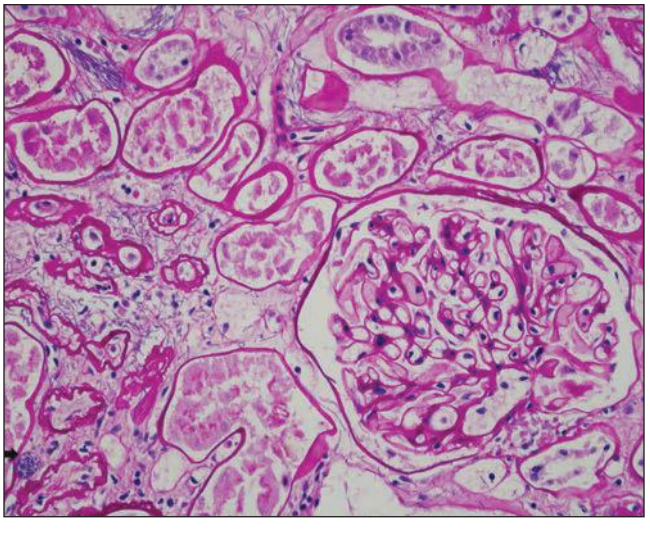


FIGURE 24.30 Renal candidiasis. Bundles of fungal pseudohyphae invade the interstitium and tubules; fungal spores are focally present (*arrow*); an inflammatory response is lacking. Autopsy kidney from a 49-year-old man who died abruptly from disseminated candidiasis involving multiple organs. (PAS; ×400.)

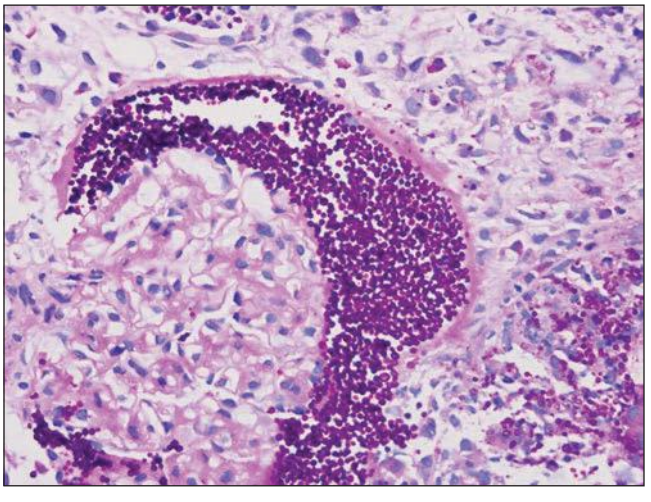


FIGURE 24.31 Renal allograft biopsy from a 50-year-old woman with end-stage kidney disease secondary to diabetes who presented with acute renal failure. Sections show PAS-positive yeast spores in Bowman space and the interstitium. Culture results indicated *Candida glabrata.* (Courtesy of Salinas-Madrigal, Saint Louis University, St Louis, MO.)

tract and mucosal surfaces, cutaneous wounds, or intravenous access lines. Renal aspergillosis is frequently the result of hematogenous dissemination, usually from invasive bronchial infection, necrotizing pneumonitis, or infarct by aspergilli. Patients receiving corticosteroids, neutropenic patients, diabetics, and immunocompromised patients (26) are particularly at risk. Less frequently, the kidneys may be involved through the ascending route (141). Renal parenchymal infections may produce symptoms comparable to those of acute pyelonephritis. The urinary tract may be obstructed by growth of mycelium, and fungus balls may be passed into the urine.

Renal involvement occurs in 30% to 40% of patients who die of disseminated aspergillosis (136). The involvement may be bilateral in systemic infections and in those with isolated renal involvement. Multiple small abscesses, a few millimeters in diameter and each surrounded by a red rim, are most common. However, extensive abscess formation with vascular invasion, thrombosis, and infarction may occur. Microscopic examination reveals inflammation with mononuclear cells and neutrophils. Abscess formation and infarcts containing typical septate branching hyphae are also seen. The fungus forms branching septate hyphae 3 to 5 μm wide best demonstrated with PAS or Grocott methenamine silver stain (Fig. 24.32). This morphology, however, is not specific to aspergillosis. Members of the genera *Fusarium* and *Pseudallescheria* show similar histomorphology.

The mechanism of Aspergillus infection involves adherence to epithelial surfaces and release neutrophil phagocytosis. Some of these released proteases may facilitate vascular invasion and thrombosis. The mechanism by which killing of *Aspergillus* takes place continues to be a subject of investigation, but there is some evidence to suggest that priming of neutrophils by formylated tripeptide followed by IL-8 enhances phagocytosis of serum-opsonized conidia from 15% to 55% (142) and distinct human monocyte subsets such as CD14(+)CD16(+) that secrete large amounts of tumor necrosis factor (TNF) are superior in fighting *Aspergillus* sp. compared to other monocytes (143).

Cryptococcosis

Cryptococcus neoformans, the agent of cryptococcosis, is a yeast-like fungus encountered in avian habitats, particularly those contaminated with pigeon dropping. The portal of entry to the host is the respiratory tract. Pulmonary infection is common, particularly in immunosuppressed and neutropenic patients. Hematogenous dissemination results in central nervous system and organ-based infection, including the kidneys. In renal transplant recipients, transmission may be donor derived (144). Renal involvement may be clinically silent or may manifest with costovertebral angle tenderness, pyuria, and gross hematuria. Yeast forms can be cultured from the urine and can be recognized in the urinary sediment by negative staining with India ink.

Renal involvement is found in about 50% of patients who die of disseminated cryptococcosis (136). Small parenchymal abscesses, or granulomas, with central necrosis involving the cortex and medulla have been described (136). The organism may elicit little inflammation despite extensive tubular destruction. Cryptococci can be identified in tissue sections as 4- to 20-μm-diameter spherical structures with a polysaccharide capsule (Fig. 24.33). The capsule is distinct, staining intensely with mucicarmine, Alcian blue, and PAS. Renal impairment has been described, but was attributed to coexistent disease.

Eliminating cryptococci from sites of infection involves growth inhibition, a process that depends on nitric oxide production by phagocytes, and phagocytosis by macrophages, a complement-dependent mechanism modulated by cytokines.

Histoplasmosis

Histoplasmosis, caused by *Histoplasma capsulatum*, is endemic in South and Central America and in the Ohio River and Mississippi River valleys in the United States (136). Infection is caused by inhalation of dust particles from soil contaminated with bird or bat droppings containing the fungus. The initial presentation resembles pulmonary tuberculosis. Disseminated histoplasmosis may result from a primary infection or from a reactivated, healed lesion during

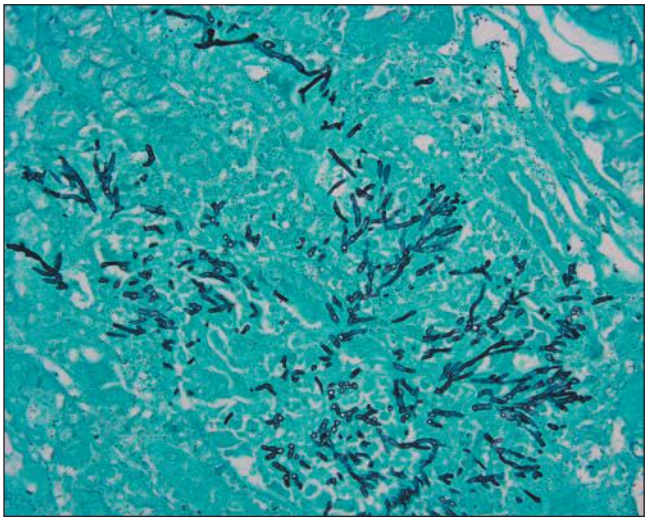


FIGURE 24.32 Renal aspergillosis. Large numbers of branching septate hyphae are illustrated. (methenamine silver × 380.)

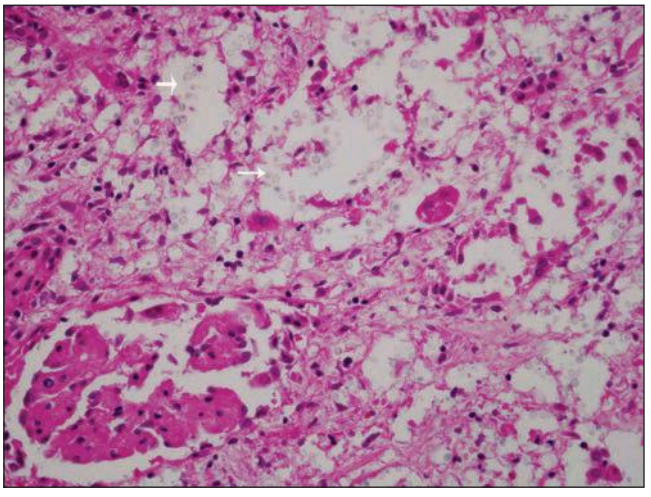


FIGURE 24.33 Renal involvement in systemic cryptococcosis. Yeast within glomeruli and tubules are separated by clear halos corresponding to their thick capsule. (H&E; ×200.)

immunosuppression. The disease is not contagious. Renal involvement is usually clinically silent, and compromise of renal function is uncommon.

The kidneys are involved in about 40% of patients as a result of progressive disseminated histoplasmosis. Less commonly, infection may be acquired during graft implantation. Grossly, lesions range from one or more well-circumscribed nodules to diffuse inflammation and necrosis. Papillary necrosis has also been described. Microscopically, small aggregates of yeast-laden macrophages may be present in all renal compartments, usually associated with granulomas. Parasitized macrophages show large numbers of round to oval yeast measuring up to 5 μm in diameter, usually identifiable with Grocott methenamine silver stain (Fig. 24.34). The histologic diagnosis of histoplasmosis can be confirmed by immunofluorescence with antibodies to polysaccharide antigens of *H. capsulatum* (136).

Coccidioidomycosis

Coccidioidomycosis, caused by *Coccidioides immitis*, is endemic in the southwest and western United States. Sporadic cases of disseminated coccidioidomycosis have been reported in nonendemic areas. In nature, the fungus is in a mycelial form, but it produces arthroconidia, which is the infective form. Inhalation of arthrospores causes symptomatic infection in about 10% of patients. Progressive pulmonary infections are rare. Systemic dissemination occurs in <1% of cases. Of these cases, patients are typically immunosuppressed, diabetic, or pregnant. Although not contagious, coccidioidomycosis is transmissible during autopsy procedures, presumably through aerosolization of endospores (145).

The kidneys are involved in one third of patients who die of disseminated infection (136). Grossly, minute granulomas and abscesses are present. The granulomas show caseous or suppurative necrosis. *Coccidioides immitis* organisms are easily found in active lesions within macrophages or giant cells as thick-walled spherules, about 100 μm in diameter, containing endospores that are 5 to 30 μm in diameter (136). Disease progression has been equated with attenuation of cellular immunity from antigen overload, suppressor cells, immune complexes, and immunosuppressive factors released from the fungus. Containment of infection depends on cellular immunity that, through release of lymphokines, enhances phagosome-lysosome fusion and killing of the fungus as described above for other fungi.

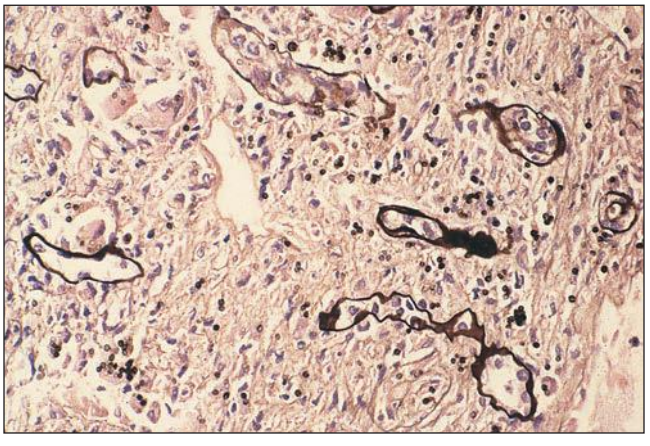


FIGURE 24.34 Histoplasmosis in a renal allograft. Yeast forms of *H. capsulatum* are highlighted with silver stain (*arrows*). (methenamine silver × 400.)

Blastomycosis

North American blastomycosis, caused by *Blastomyces dermatitidis*, is endemic in the Ohio and Mississippi River valleys and the southeastern United States (136). The fungus is a saprophytic budding yeast found in soils. Infection results from inhalation of infectious forms. Blastomycosis is prevalent among immunosuppressed patients. It is found four times more frequently in males than in females. Infection typically occurs between the ages of 30 and 50 years (136). Primarily, a pulmonary infection disseminates through blood to various organs, including the kidneys. Renal involvement is usually clinically silent. In severe infections, fever, weight loss, chest pain, cough, costovertebral angle tenderness, flank pain, renal insufficiency, and chronic discharging sinuses or subcutaneous abscesses have been reported. The diagnosis can be established through culture or by identifying the organism in fluids or tissue sections. Blastomycosis is not a transmissible disease.

Renal blastomycosis is estimated to occur in 25% of systemic infections. Involvement is often bilateral and varies from small, circumscribed nodules to diffuse inflammation and necrosis (136). The cortex is more often affected than the medulla. Perinephric abscesses and discharging sinuses may result from extension of the infection through the capsule. In tissue sections, granulomatous and suppurative lesions are seen. Occasional microabscesses and epithelioid caseating granulomas form, resembling tuberculosis. *Blastomyces dermatitidis* can be detected in either type of lesion as yeast cells with a double-contoured appearance. The cells measure 8 to 15 μm in diameter and demonstrate broad-based budding. Although organisms can easily be seen in routine hematoxylin and eosin–stained sections, the Grocott methenamine silver stain facilitates their detection. Antibodies against the cell wall polysaccharide antigen of *B. dermatitidis* are available and can be used for organism identification (136).

Paracoccidioidomycosis

Paracoccidioidomycosis (i.e., South American blastomycosis), caused by *Paracoccidioides brasiliensis*, is a chronic pulmonary disease endemic to Mexico and South and Central America. Pulmonary infection caused by inhalation of spores from *P. brasiliensis* tends to be progressive and followed by dissemination to mucous membranes, lymph nodes, and various organs. Although the presenting symptoms are usually pulmonary, the disease is often manifested by dissemination.

The kidneys are involved in 10% to 15% of cases of disseminated infection. Grossly, the renal cortex and medulla contain miliary necrotizing granulomas measuring a few millimeters (136). Organisms are found at the periphery of necrotic granulomas and within giant cells, easily identified in sections stained with hematoxylin and eosin. The Grocott methenamine silver stain brings out greater detail. Nonbudding forms, about 10 μm in diameter, predominate. Multiple buds from a single cell, 10 to 60 μm in diameter, are diagnostic, but less common. These resemble the steering wheel of a ship (136).

Mucormycosis

Mucormycosis (i.e., zygomycosis) is an opportunistic infection of the lungs and upper respiratory tract caused by fungi of the order Mucorales, whose most common pathogen is *Rhizopus oryzae*. Infection is acquired by inhalation of airborne spores. Disseminated infection occurs in immunocompromised and diabetic patients. Rarely, mucormycosis may only involve the kidney. *Rhizopus oryzae* frequently invades blood vessels and disseminates through the blood. Morrison and Mcglave (146) reported mucormycosis in 13 of 1500 bone marrow transplant recipients (0.9%), with kidney involvement in a single patient. Involvement of the urinary tract may be clinically silent or show signs of renal infection, including flank pain, dysuria, gross hematuria, and acute renal failure (136).

Renal involvement occurs in 50% of patients dying of disseminated mucormycosis (136). Thrombosis may occur, resulting in segmental or subtotal renal infarction. Involvement can be unilateral or bilateral. Microscopically, there is suppurative, necrotizing inflammation with thrombosis of interlobar and arcuate arteries. Granulomatous inflammation, fibrosis, and Langerhans-type multinucleated giant cells are seen. Hyphae can be detected in areas of acute inflammation or infarction. In tissue sections, the fungus has broad, nonseptate hyphae with right-angle branching (Fig. 24.35). Organisms can be identified with Grocott methenamine silver stain or fluoresceinated antibodies (136).

Viral Nephropathies

Viral Pathogenesis and Tropism in the Kidney

When encountering a host, eukaryotic cell viral pathogenesis requires an initiation phase in which the virus must first cross the cell membrane and enter the cell usually through a receptor-mediated process. The virus must then cross the nuclear envelope and enter the nucleus. During the replication phase, DNA synthesis, transcription, and translation of viral genes occur. Finally, during the release phase, assembly and maturation precedes the exit of the virus from the cell. Viruses have evolved several subversion mechanisms at all of these phases, which require the large-scale use of the host cellular machinery. Bruggeman (147) identifies and gives examples of the subversion mechanisms viruses use in chronic kidney disease (CKD) as (a) molecular mimicry, (b) hijacking strategies, and (c) transformers and oncogenes. Viruses must also evade the host immune response.

Cytomegalovirus (CMV) uses extensive molecular mimicry to avoid the host immune response. Of the 160 genes encoded by CMV, the majority are employed to manipulate the host immune system by mimicking cytokines, chemokines, chemokine receptors, and cytokine-binding proteins. As an example of immune molecular mimicry, CMV produces an IL-10 homologue (vIL-10), which suppresses the host pro-inflammatory cytokines and helps the virus evade the host immune response.

HIV-1 can "hijack" the NFκB signal transduction pathway in both immune and kidney cells. When IkB is phosphorylated and degraded, its inhibition of NFκB is removed, and NFκB can translocate to the nucleus and activate transcription. The net result is not only the vigorous transcription of viral genes but also activation of NFκB-dependent host genes, which leads to abnormal regulation of processes such as proliferation and apoptosis in the host kidney cells and may contribute to the pathogenesis of HIV-associated nephropathy (HIVAN). These altered processes yield a nonmalignant transformation of kidney epithelial cells, which in HIVAN may be represented morphologically by visceral epithelial hyperplasia and tubular ectasia.

Viruses can play a role in inducing cellular mechanisms of kidney injury and involve the kidney as an "innocent bystander" though a number of mechanisms (148). For example, TLRs on kidney dendritic cells can recognize virally

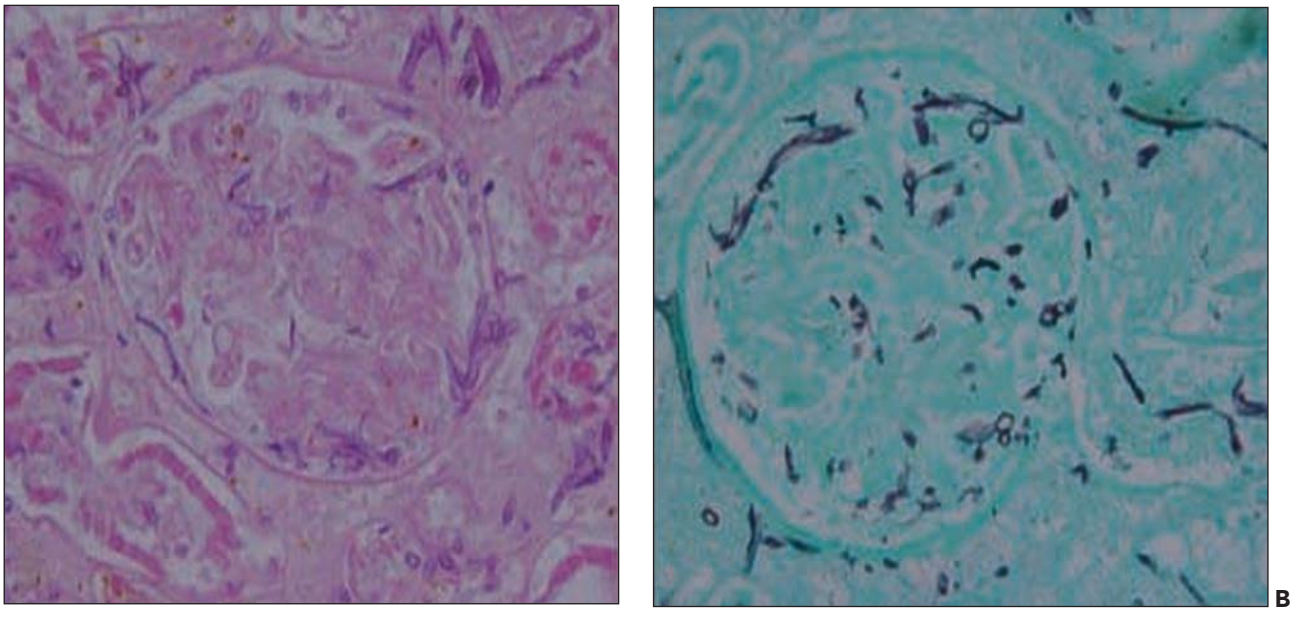


FIGURE 24.35 Renal mucormycosis involving glomeruli. A: Hyphae are broad and nonseptate with right-angle branching. (H&E; ×400.) **B:** Methenamine silver × 400. (Courtesy of Antony Chang University of Chicago, Chicago, Illinois.)

encoded molecules and facilitate a TH1 response at the locale of the renal parenchyma. The subsequent inflammatory response may injure the kidney. As a phenotypic disease example of virally induced innocent bystander damage, the hemophagocytic syndrome (HPS), in which nonmalignant proliferations of activated macrophages infiltrate many organs, can cause acute renal failure. Viral triggers such as Epstein-Barr virus (EBV), parvovirus, and CMV have been described in HPS. Heterologous immunity, in which established memory T-cell responses to a previously encountered pathogen, can have a major impact on the course and outcome of a subsequent infection with an unrelated pathogen. Heterologous immunity is dependent on the sequence of infections, the prior T memory network at the time of the infection, and can be either beneficial or detrimental to the host in transplantation settings (149).

Viruses may also perturb the T-lymphocyte–dependent B-lymphocyte response with subsequent B-cell hyperactivity and a loss in tolerance through mechanisms such as molecular mimicry and epitope spreading (150). In these situations, autoimmune or alloimmune antibodies are produced and may facilitate autoimmune disease or rejection.

Viral tropism to the kidney is determined by both virus and tissue factors. In cell culture, human CMV has differential tropism for primary human cells and cell lines (151). Differences in virus cell-binding factors may also influence tropism to the kidney. Once again with CMV, in a murine CMV model, homologues of the human CMV tegument phosphoprotein pp65, when mutated, will exhibit differential tropism among the liver, spleen, salivary gland, and kidney (152). The localization factors of virus and kidney cells impacting human disease are largely unexplored.

The Biology of Epstein-Barr Virus Infection

EBV infections in nonimmunosuppressed hosts are common. Most adults have serologic evidence of past infection. Primary infections may be manifested as infectious mononucleosis. Primary infections may also be silent with little in the way of clinical signs and symptoms. The major target for EBV is the B cell. EBV attaches to cells for which it is tropic through the CD21 receptor. Augmented GP110, which is the product of the EBV gene BALF4, may dramatically enhance viral tropism (153). In infected cells, the EBV virus may exist in a latent or a lytic state. A latently infected B cell may exist in one of four programs (154). Latency "0" is characterized by complete silencing of the viral genome. In healthy carriers of EBV, between 1 and 50 per million peripheral blood lymphocytes are infected. The infected cells of latency "0" are phenotypically similar to long-lived memory cells. Latency I expresses LMP2A (late membrane protein) alone or together with EBNA-1 (EBV nuclear antigen). In latency II, EBNA-1 and the three LMPs (LMP1, LMP2A, and LMP2B) are expressed in infected B cells that home to the germinal centers. In the latency III program, all nine of the latency genes (EBNA-1, EBNA-2, EBNA-3 [or EBNA-3A], EBNA-4 [or EBNA-3B], EBNA-5 [or LP], and EBNA-6 [or EBNA-3C] and the latent membrane proteins LMP1, LMP2A, and LMP2B) are expressed. Latency III is the "growth program," which is associated with autonomous B-cell growth. The latency III program is expressed in the immunoblast-like cells of posttransplant lymphoproliferative disorders.

During early latency, EBNA-2 activates B lymphocytes through induction of CD23. Also in latency, the EBV LMP induces expression of CD23, ICAM1, and LFA-3. The highest-frequency RNA in late latency is EBER. Latently infected cells can be switched into a productive or lytic phase by a number of environmental stresses. The immediate early gene of the latent to lytic switch is BZLF-1. GP-350 is expressed in the late lytic phase. EBV mutants defective for lytic viral replication are unable to promote the induction of posttransplant lymphoproliferative disorder (PTLD) in mouse models, a phenomenon that has been ascribed to the decreased production of IL-6, IL-10, and angiogenic factors, such as VEGF (154,155). Thus, there may also be a significant role of lytic infection in the pathogenesis of PTLD.

Posttransplant Lymphoproliferative Disorders and EBV Infection

In latently infected immunocompetent hosts, B-cell proliferation is kept in check by cytotoxic T cells that recognize and kill infected B cells. In patients with a compromised T-cell immune system, EBV-activated B cells may proliferate without regulation, resulting in B-cell expansions and neoplasia. Posttransplant lymphoproliferative disorders are lymphoid proliferations, which occur in immunosuppressed patients including bone marrow and solid organ transplant recipients, patients treated with immunosuppression for autoimmune disease, and patients with inherited immune deficiencies. Longitudinal studies in PTLD patients show a progression from the restricted pattern of latency "0," which is seen in healthy carriers to the broader patterns of EBV latency III coupled with lytic replication (156).

Among solid organ transplant recipients, patients receiving renal allografts have the lowest frequency of PTLD at <1%. Cardiac allografts (1% to 2%) and heart-lung or liver-bowel (5%) having increased frequencies of PTLD. Over 80% of PTLD cases are associated with EBV infection. In solid organs, most PTLD cases are of recipient cell origin with <10% of donor origin. This suggests a role of endogenous EBV reactivation in many of these patients. There are multiple risk factors for PTLD. The most important is seronegativity for EBV, which conveys a 24X risk. Other risk factors include CMV seromismatch and prior therapy with OKT3. A patient with all of these factors is estimated to have a 654X risk over the reference transplant population for the development of PTLD. Serologic testing for EBV is unreliable in immunosuppressed patients. In general, EBV viral loads are increased in PTLD. Many studies show elevated EBV viral loads as measured by PCR of blood (157,158). There is controversy over the standardization and reference ranges in viral load testing (159,160).

Frequent molecular changes in PTLD involving cellular genes include alterations of c-MYC, BCL-6, p53, aberrant promoter hypermethylation, and somatic hypermutations targeting multiple protooncogenes (161).

Pathology of PTLD

The lesions of PTLD are categorized by the World Health Organization (162) according to Table 24.3. Since some of the histopathologic features of rejection can overlap with PTLD in kidney tissue, particular attention to the morphology of "early lesions" and polymorphous PTLD is warranted. "Early lesions" are characterized by either marked plasmacytosis (in the absence of substantial chronicity) or an infectious mononucleosis–like reaction with a brisk lymphoid proliferation showing a mixture

TABLE 24.3 World Health Organization categories of PTLD

- **Early lesions**
  - Plasmacytic hyperplasia
  - Infectious mononucleosis–like
- **Polymorphic PTLD**
- **Monomorphic PTLD**
  - B-cell neoplasms
    - Diffuse large B-cell lymphoma
    - Burkitt/Burkitt-like lymphoma
    - Plasma cell myeloma
    - Plasmacytoma-like lesions
  - T-cell neoplasms
    - T/natural killer cell lymphomas
    - Peripheral T-cell lymphoma
    - Hepatosplenic T-cell lymphoma
- **Classic Hodgkin lymphoma**

of small lymphoid cells, plasma cells, and rare immunoblasts (Fig. 24.36). In "polymorphous PTLD," the mixed morphotype of the infectious mononucleosis–type reaction is repeated but with a greater number of immunoblasts, occasional atypical immunoblasts, cells with irregular nuclei resembling centrocytes, increased mitoses, and frequently, necrosis (Fig. 24.37). PTLD involving renal allografts does not show the lymphocytic tubulitis or vasculitis of cellular rejection.

Renal Involvement in PTLD

In a representative series of 36 renal transplant (31 cases) and/or renal/pancreas (4 cases) transplant patients with a diagnosis of PTLD from the University of Pennsylvania (163), PTLD was diagnosed from 6 days to 10 years after engraftment (mean 509 days). Fourteen patients (40%) had been given 10- to 20-day courses of OKT3 or ALG prior to PTLD diagnosis. All 14 of these patients developed PTLD within 6 weeks of transplantation. Seven of ten patients tested showed recent

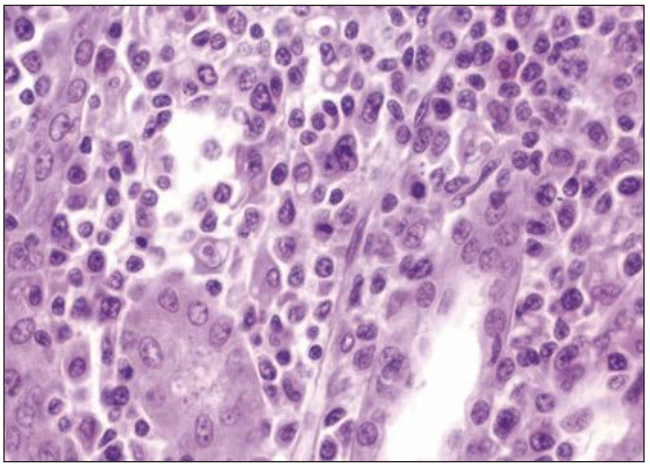


FIGURE 24.36 Early mononucleosis–like infiltrate of PTLD. The interstitial infiltrate is mostly mature lymphocytes. Only rare immunoblasts are seen. (H&E; ×27.)

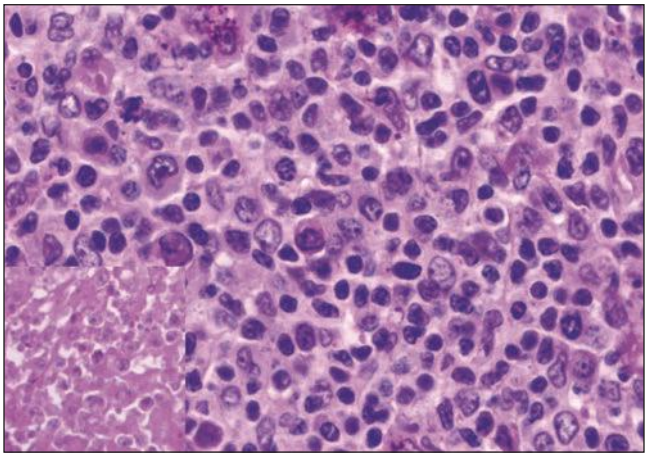


FIGURE 24.37 Polymorphous PTLD. The number of transformed cells is increased, with many immunoblasts. Focal necrosis is seen **(inset)**. (H&E; ×27.)

EBV infection, and 7 of 15 patients tested had active CMV infection at the time of PTLD diagnosis.

Essentially all of the morphologies listed in Table 24.3 have been described in renal allografts. The recognition of the early lesions and polymorphic histologies of PTLD are particularly critical to the practicing nephropathologist in that the distinction from rejection is challenging. Furthermore, the therapeutic approaches to rejection and PTLD are markedly different making the distinction between the two conditions all the more important. In the University of Pennsylvania series (163), the initial PTLD diagnosis was classified as "early" lesion (13 patients) polymorphic PTLD (P-PTLD) (11 patients) and monomorphic PTLD (M-PTLD) (12 patients). Thirty-three lesions were B-cell proliferations, while the remaining two lesions were gamma-delta T-cell lymphomas. Two patients who initially presented with early lesions rapidly progressed to P-PTLD and then to M-PTLD. Thirty-two of the PTLDs, all of which were B cell in phenotype, were EBV positive by in situ hybridization. Seventeen patients (49%) presented with PTLD in the allograft. Of the 17 patients that presented with allograft PTLD, 9 showed moderate to marked interstitial hemorrhage. Six of these patients had renal biopsies prior to PTLD diagnosis. The renal biopsies prior to PTLD were all classified as moderate to severe acute rejection. While these biopsies showed acute rejection (tubulitis, vasculitis, and/or interstitial hemorrhage), closer examination also showed transformed lymphocytes, lymphocyte mitoses, and occasional immunoblasts in three patients. In situ hybridization showed EBV in these three biopsies. Thus, very early PTLD in allografts may coexist with rejection.

PTLD can present in renal allografts and may be confused with allograft rejection. Careful examination of the histology and EBV studies may be needed to distinguish PTLD from rejection. Allograft biopsies with dense lymphoid infiltrates should be evaluated for transformed lymphocytes, lymphoid mitoses, and immunoblasts. These findings may precede PTLD development. Allograft involvement by PTLD is also often associated with interstitial hemorrhage, a finding which is also seen in humoral rejection. Further analysis

for PTLD, including studies for EBV and C4d, should be undertaken in renal allograft recipients with dense lymphoid infiltrates showing atypia and/or interstitial hemorrhage.

Most cases of PTLD in renal transplants regress with reduction of immunosuppression; however, a minority of cases can progress to lethal lymphoma. The time from infection to fatal disease progression can be as little as a few weeks. The early recognition and diagnosis of PTLD can offer an opportunity for reduction of immunosuppression and reconstitution of the host immune system, which is necessary for control of the EBV infection.

Cytomegalovirus Nephritis

20 to 60% of renal transplant recipients were reported in the literature to suffer from CMV disease with clinical signs of fever, leukopenia, and organ dysfunction prior to 2000 (164,165). However, the disease is rare today after prophylactic and preemptive CMV therapies were implemented (166). CMV infection in a renal allograft recipient may be a primary infection, a reactivation infection, or a reinfection. Primary infections occur in previously uninfected patients who are seronegative for CMV. Blood products or the donated allografts are the usual sources of infection. Reactivation infection occurs in previously infected recipients who reactivate latent infections. Reinfection occurs when a seropositive recipient acquires a new strain of latent virus from a seropositive donor with subsequent reactivation. The most significant risk factor for primary CMV infection is receipt of a seropositive donor allograft by a seronegative recipient (165). Children are at the highest risk for primary infection. The extent of immunosuppression is the other risk factor for CMV infection. The total amount of immunosuppressive therapy and, in particular, use of antilymphocyte antibodies enhance the risk for CMV infection. Studies of the viral gene products targeted by the host immune responses have demonstrated that the human CMV 65-kDa tegument phosphoprotein pp65 (UL83) is the target of antibody (167), cytotoxic T-lymphocyte (CTL) (168), and lymphoproliferative (169) responses.

The histopathologic changes associated with CMV infection vary. Cowdry type A intranuclear CMV inclusions are the typical (CMV is the only virus to show intranuclear Cowdry type A and intracytoplasmic inclusions) and diagnostic finding, but interstitial inflammation, glomerulopathy, ATN, and other findings pose diagnostic dilemmas. A seminal article by Richardson et al. (170) described a glomerulopathy in renal allografts with endothelial swelling, hypertrophy and necrosis, obliteration of the capillary lumens, fibrillar deposits in the glomerular capillaries, mild segmental hypercellularity, mononuclear cell infiltration, but absent CMV inclusions. Since most of these patients had CMV viremia or CMV infection without viremia, the authors associated this pathology with CMV infection and not rejection. Tuazon et al. (171) described similar cases and noted a predominance of CD8+ cells in the glomeruli. Rao et al. (172) described a case of de novo immunotactoid glomerulopathy with resolution after recovery from a CMV infection. Others have questioned the causative role of CMV infection in such cases. Herrera et al. (173) in studying immunosuppressed patients with CMV infection found histologic lesions similar to those previously described; however, no parenchymal evidence for CMV infection of the kidney could be found by immunofluorescence or electron microscopy. The authors raised the possibility of damage secondary to antiendothelial antibodies and vascular rejection. Previous studies by Anderson et al. (174), using immunohistochemistry and in situ hybridization from CMV viremic allograft recipients with glomerulopathy, could find no evidence of CMV antigens or DNA. Rubin (175) has suggested that CMV-mediated injury may be indirect. Proinflammatory cytokines such as gamma interferon can up-regulate MHC in kidney. CMV infection may also increase both class I and II MHCs. Immunologically mediated injury resulting from enhanced MHC expression and increased targeting may produce the lesions associated with CMV glomerulopathy.

A second mechanism for CMV damage of kidney is more clearly related to direct viral infection. Payton (176) described CMV inclusions in glomerular and peritubular capillary endothelial cells as well as in tubular epithelial cells (Fig. 24.38). Cameron (177) described CMV inclusions in tubular epithelial cells in the absence of rejection. Birk and Chavers (178) reported CMV inclusion glomerulopathy in a young transplant patient. Such cases, while rare, document productive CMV infection in both glomerular and tubulointerstitial compartments in a small subset of patients. CMV infection may be diagnosed by serology, tissue examination through histology with immunofluorescence or immunohistochemistry, or CMV-PCR of buffy coat or tissue. Quantitative buffy coat CMV-PCR does not correlate well with the tissue presence of CMV inclusions; however, the frequency of discovering CMV infection in renal allografts may be increased by CMV-PCR techniques (179).

Adenovirus Infection

Adenoviruses belong to the family *Adenoviridae*, which include a group of DNA viruses such as polyoma viruses and herpes viruses. Adenoviruses are double-stranded DNA viruses. Adenovirus can cause respiratory infection, pharyngitis, keratoconjunctivitis, gastroenteritis, hepatitis, hemorrhagic cystitis, and nephritis. In immunocompetent hosts, many adenovirus infections are subclinical. Significant infections with

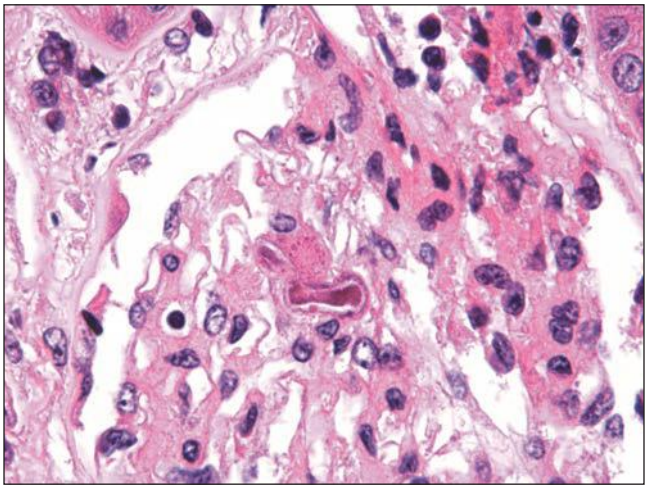


FIGURE 24.38 Cytomegalovirus glomerulitis with productive infection, as demonstrated by classic intranuclear and cytoplasmic CMV inclusions. (H&E; ×67.)

adenovirus are well described in immunosuppressed patients including solid organ and bone marrow transplant patients and may present as disseminated infection (180). Adenovirus infection may also present as acute hemorrhagic cystitis in renal transplant patients (181). Adenovirus viremia is cited in approximately 7% of renal transplant patients (182), and the virus is excreted in approximately 11% (183). Serotypes 7, 11, 34, and 35 constitute most of the cases (184). Adenovirus has tropism for epithelial cells via coxsackievirus and AdV receptors, class I human leukocyte antigen molecules, and sialoglycoprotein receptors (185); CD46; and fiber knob gene protein (186). Secondary interactions with integrins may be needed for virus internalization.

Most adenovirus-associated infection in kidney transplants occurs within months of engraftment. It is thought that the majority of these infections are reactivation infections, but late de novo (187) and early donor-derived infection (188) cases are reported. Patients with nephritis present with hematuria, fever, and renal dysfunction. Occasionally, mass lesions are seen on imaging (189). Urine PCR can be used to obtain a rapid confirmation of UTI (190), but distinction between cystitis and nephritis is problematic and can only be made with biopsy. The pathologic changes in the kidney are those of an acute interstitial nephritis with lymphocytes, histiocytes, plasma cells, and occasional neutrophils. Granulomatous inflammation and necrosis can also be seen. Necrotic tubular epithelial cells may be frequent. The distal nephron is primarily affected, and interstitial nephritis is reported as being maximal in the medulla and at the corticomedullary junction. The differential diagnostic considerations include rejection, BK virus infection, interstitial nephritis secondary to other infections (i.e., fungus, TB), and drugs. The characteristic cytopathic effect of adenovirus infection is the "smudge cell," which is found among degenerated tubular nuclei and consists of an amphophilic or basophilic intranuclear inclusion body filling the enlarged nucleus with indistinct nuclear borders and smudged chromatin (Fig. 24.39). Parenchymal necrosis, neutrophil infiltrates, tubular red blood cell casts, and focal interstitial hemorrhage are also seen with adenovirus infection and are not typical for other allograft viral infections. Confirmation of adenovirus infection may be morphologically done by electron microscopy, immunohistochemistry, or in situ hybridization. On ultrastructural exam, virions are nonenveloped, have a hexagonal outline, are 70 to 110 nm in diameter, and are aggregated in a crystalline array. Both cytoplasmic and nuclear labels are seen by immunohistochemistry (191). Disseminated disease has a high mortality in renal transplant patients. The relationship between viral infection and rejection is controversial (192).

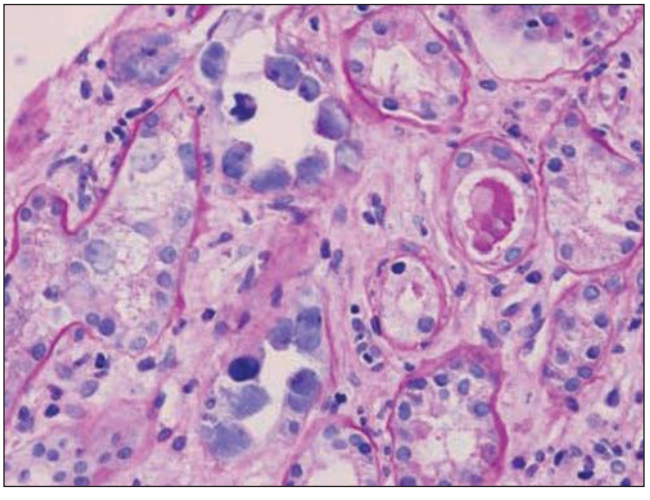


FIGURE 24.39 Renal involvement by adenovirus demonstrates characteristic smudge cells. (H&E; ×400.)

Hantavirus Infection

Hantaviruses belong to the family Bunyaviridae. Hantaviruses chronically infect rodents and spread by aerosolized excreta to humans. There are two main syndromes associated with clinical Hantavirus infection, that is, hemorrhagic fever with renal syndrome (HFRS) and Hantavirus pulmonary syndrome (HPS). We will focus only on HFRS in this section (193).

HFRS is dominant in Asia including China, Korea, and eastern Russia with more than 100,000 cases annually caused by Hantaan virus carried by the striped field mouse (*Apodemus agrarius*). Dobrava virus carried by the yellow-necked field mouse (*Apodemus flavicollis*) is the usual cause of severe HFRS in the Balkans. Puumala virus carried by the bank vole (*Clethrionomys glareolus*) is found in Scandinavia, Western Russia, and the Balkans and causes less severe disease. Seoul virus from the gray rat (*Rattus norvegicus*) is worldwide in distribution. In the United States, the most likely causative virus of HFRS is the Seoul virus. The incidence of HFRS in the United States is not well known. The prevalence of positive serum antibodies for Seoul virus in urban US residents is <1%.

Clinically, HFRS is typically divided into four phases: a febrile period lasting 3 to 5 days, a hypotensive phase lasting a few hours to 2 days, an oliguric phase lasting a few days to 2 weeks, and a polyuric recovery phase (193). Symptoms include fever and chills, myalgias, nausea and vomiting, thirst, abdominal pain, photophobia, and periorbital edema. Petechiae, thrombocytopenia, and disseminated intravascular coagulation are common. There is an extensive vascular leak syndrome with hemoconcentration and postural hypotension. Back pain may be associated with retroperitoneal fluid accumulation. Severe hypotension and shock follows. Proteinuria is marked, and urine specific gravity falls followed by oliguria. The more severe cases of Hantaan virus infection in Asia or Dobrava virus infection in the Balkans have case fatality rates of 5% to 15%. The less severe HFRS from Seoul or Puumala virus infection usually do not display the full spectrum of clinical manifestations and have a case fatality rate of <1%. HLA associations with severe disease have been described (194).

Physiologically, the acute phase of HFRS is characterized by a markedly decreased GFR and increased glomerular permeability with impairment of both the size- and charge-selectivity properties of the glomerular filter (195).

The most common renal findings in acute Hantavirus infection are acute interstitial nephritis with dominant lymphocytic inflammation accompanied by acute interstitial hemorrhage and tubular necrosis (196,197). No viral inclusions are present. Biopsies taken later in the course may show interstitial fibrosis. Glomerular changes in these cases are limited to some mild mesangial hypercellularity. A small number of cases show diffuse proliferative glomerulonephritis (198), sometimes

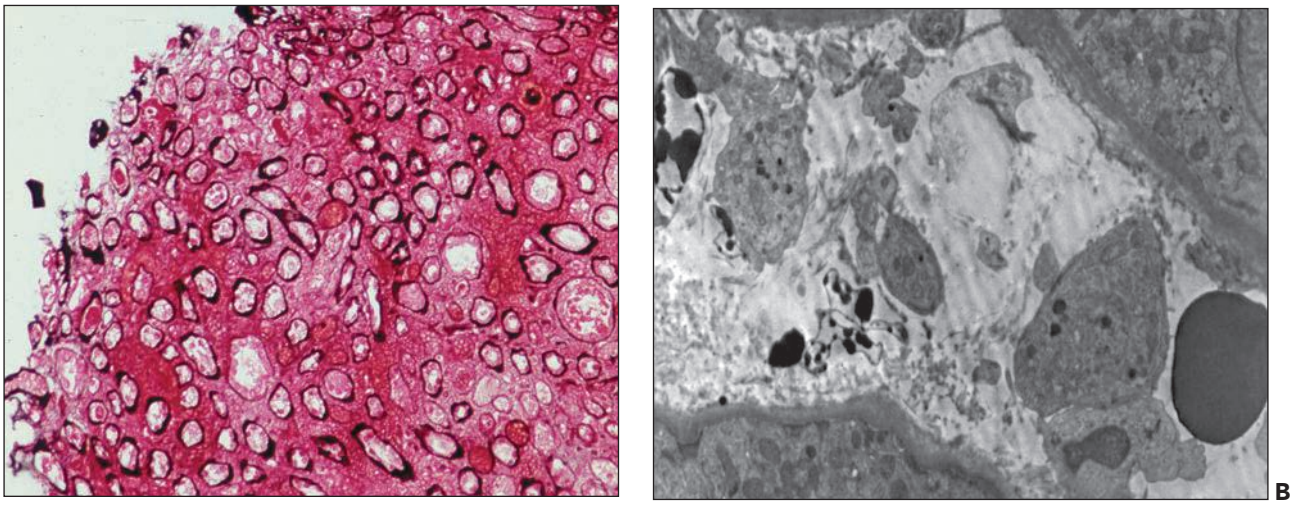


FIGURE 24.40 Hantavirus virus pathology in renal biopsy from a 30-year-old man who presented with flu-like symptoms following contact with rodents. A: There is a sea of red blood cells (hemorrhagic nephritis) in the interstitium particularly in the medulla. **B:** Endothelial cell injury causes edema in peritubular capillaries better appreciated with electron microscopy. (Courtesy of Drs. Dusan Ferluga and Alenka Vizjak, University of Ljubljana, Slovenia.)

with a pattern of MPGN (199). The typical biopsy pathology of Hantavirus nephropathy (200) is characterized by massive acute interstitial hemorrhage particularly in the outer stripe of the outer medulla (Fig. 24.40). The interstitium has dilated peritubular capillaries filled with blood and massive interstitial hemorrhage compressing tubules. Both intact and dysmorphic red blood cells are seen extravasated in the interstitium. There is a sparse interstitial infiltrate composed of T cells and macrophages. The fine ultrastructure of the family Bunyaviridae (201) is that of a round or oval in shape, about 100 nm in diameter, with a two-layer lipid envelope from which spikes protrude. The infected cells display an enlarged and proliferating Golgi apparatus.

Interferon is thought to play a role in the cellular defenses against Hanta virus infection at an early stage (202). The major pathogenetic mechanisms may be related to the immune system's response to the virus. In part, the damage in Hantavirus disease results from the elimination of the virus with subsequent necrosis. T-cell–mediated responses including the T-cell cytokines TNF-α and γ-interferon are prominent in Hantavirus disease. Hantavirus is endotheliotropic, and viral particles are found in the endothelial cells of the kidney particularly in the outer medulla (203). Increased expression of TNF-alpha, as well as intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and platelet-endothelial cell adhesion molecule-1, is present in the peritubular areas of the distal nephron (204).

Other Viral-Induced Hemorrhagic Fevers With Acute Kidney Injury

Viral hemorrhagic fevers (VHFs) are diseases caused by the RNA virus from four different families, namely, *Flaviviridae, Arenaviridae, Bunyaviridae*, and *Filoviridae* (205). Dengue virus and yellow fever virus belong to the family *Flaviviridae*. These infections are acquired through the bite of an infected arthropod or by the inhalation of particles of rodent excreta. Yellow fever and dengue fever are the most prevalent in tropical regions worldwide. The clinical presentation is characterized by fever, malaise, increased vascular permeability, and coagulation defects that can result in bleeding. AKI is an uncommon complication in most cases of VHF. In severe cases of yellow fever, there is tubular necrosis (206,207) without significant inflammation.

Dengue virus may cause a direct renal injury in the absence of hypotension, rhabdomyolysis, or hemolysis. Kidney injury with proteinuria and hematuria has been described. A glomerulopathy characterized by hypertrophy and hyperplasia of mesangial and endothelial cells, monocyte-like cells in some of the glomerular capillary lumens, and focal thickening of the glomerular basement membrane is reported (208).

Parvovirus Infection

Parvovirus B19 is a small nonenveloped single-stranded DNA virus, which targets erythroid progenitor cells in the bone marrow. The virus gains entry to these cells by binding to the blood group P antigen. Parvovirus infection has a well-established association with fifth disease, hydrops fetalis, transient aplastic crisis, and chronic pure red blood cell aplasia in immunocompromised patients. Many other conditions such as myocarditis, rheumatological conditions, vasculitis, a variety of neurologic diseases, interstitial lung disease, and renal disease have been linked to parvovirus infection by temporal relationship of these clinical conditions with serologic evidence of infection. The renal disease associations are in several situations (209). Acute nephritis with hypocomplementemia following fever, rash, and arthritis is reported with focal proliferative or mesangial proliferative glomerulonephritis with subendothelial deposits. B19 antigen has been demonstrated by immunohistochemistry in some cases (210). Parvovirus infection has been reported in patients with sickle cell disease (211). Moudgil et al. (212) found a higher prevalence of B19 DNA in renal biopsies from patients with collapsing glomerulopathy. In situ hybridization detected B19 in the majority of biopsies that tested positive

for virus by PCR. B19 DNA was localized to glomerular podocytes and parietal epithelial cells. Others have been unable to demonstrate viral DNA for parvovirus in cases of collapsing glomerulopathy. Acute proliferative glomerulonephritis was also described in association with B19 (213). Parvovirus and anemia are not uncommon in renal transplant patients (214).

Coxsackievirus Infection

Coxsackievirus belongs to the genus Enteroviridae. Coxsackie is an RNA virus. Coxsackieviruses are small particles measuring approximately 30 nm. Coxsackie A and B have different tropisms. Coxsackie B enters the organism through enterocytes and spreads to other organs through the blood. Most coxsackievirus infections are flu-like febrile illness, or they are asymptomatic. Coxsackie B can cause aseptic meningitis, acute and chronic myocarditis, paralytic diseases, rhabdomyolysis, pleurodynia, and severe septic diseases in newborns.

Experimental models of coxsackievirus infection produce histologies resembling MPGN or IgA nephropathy in mice. Coxsackie B virus can infect human mesangial cells. From these findings, one might expect an association of this virus with human renal disease. The literature on coxsackievirus infection and nephritis is, however, limited. Case reports suggest interstitial inflammation and/or chronic glomerulonephritis in association with coxsackievirus (215). Much more study is needed.

Kidney Injury in Influenza A Infection

AKI is reported in influenza A (H1N1) infection. The frequency varies by report. A prospective study by Pettila et al. (216) cites approximately a third of patients with severe H1N1 infection will suffer from AKI with approximately 5% requiring renal replacement therapy. The cause(s) of AKI in these patients is unclear. Rhabdomyolysis and multiorgan injury are often cited. There is very little literature on the pathology of AKI in H1N1. Nin et al. (217) report a pattern of distal ATN with epithelial cell swelling, individual cell necrosis, and shedding of epithelial cells into the tubular lumens. These authors identify immunoreactivity for viral nucleoprotein in tissue section. Jain et al. (218) reported pediatric H1N1 infection with renal failure and hematuria leading to a presumptive clinical diagnosis of postinfectious glomerulonephritis. Tissue confirmation was not obtained.

Syphilis

Syphilis is a sexually transmitted disease, currently underestimated and underrecognized. However, the Centers for Disease Control estimated about 55,000 new cases occurring in the United States alone (219). Kidney involvement commonly comes as a surprise, and the most common manifestation is glomerular involvement with secondary membranous glomerulonephritis. Most cases currently reported are case reports (220,221). These patients typically present with nephrotic syndrome but also with unusual presentation such as acute renal failure (219). Tubulointerstitial disease is rare. Tubulointerstitial nephritis including a large number of plasma cells and associated interstitial fibrosis was reported by Rich in 13 of 200 patients with documented tertiary syphilis (222). It is also important to consider syphilis in patients with HIV who show membranous glomerulonephritis on renal biopsy, particularly since it is reversible (223).

Endothelial cells are probably the initial site of infection by *Treponema*. Attachment of spirochetes to endothelial cells is facilitated by fibronectin molecules bound to the surface of the microorganism (224). The spirochetes elicit humoral and cell-mediated immune responses. The mononuclear cell infiltrate has characteristics of a delayed-type hypersensitivity reaction. Animal studies suggest this response is more important than antibodies in containing the initial infection (224). Antibodies and complement (C3) opsonize the bacterium and facilitate phagocytosis by macrophages, which kill and degrade the microorganisms. Lymphocytes also secrete soluble factors that kill *T. pallidum*.

Actinomycosis

Actinomycosis is an opportunistic infection caused by *Actinomyces* organisms, filamentous bacteria that reside in the mouth and throat of healthy individuals. The most common agent is *A. israelii*. Systemic infection results from penetration of mucosal defects usually associated with other bacterial infections. Kidney involvement is rare. In Brown's series (225) of 181 patients with actinomycosis, disseminated infection occurred in 18 patients and renal involvement in one patient. Renal involvement also may result from contiguous spread from abdominal infection (226), may be confused with neoplasia, or may cause renal vein thrombosis (227). Renal infection results in suppurative, necrotizing pyelonephritis, with multiple abscess formation. Actinomycosis is recognized by the presence of sulfur granules in fluid draining from a sinus tract or by intraoperative biopsy. Sulfur granules can easily be seen in sections stained with hematoxylin and eosin. The filaments, about 1 μm in diameter, are Gram and methenamine silver stain positive. They also can be identified by fluoresceinated antibodies to *Actinomyces*.

Nocardiosis

The most common agent of nocardiosis is *Nocardia asteroides*, a filamentous bacteria present in nature. However, the incidence of infections with *N. farcinica* is rising, and this agent, indistinguishable from *N. asteroides* by routine laboratory methods, is not sensitive to most antimicrobial agents. The bacteria are found in soils, and infection results from inhalation of infective forms or their introduction into soft tissue through trauma. Kidney involvement is rare and occurs as part of hematogenous dissemination, usually a pulmonary infection in immunocompromised patients (228) or in patients receiving corticosteroids. Hematogenous dissemination may be complicated by vascular thromboses (229). Renal infection results in single or multiple small abscesses or diffuse pyelonephritis, often with draining sinus tracts (229). Identification of *Nocardia* must rely on Gram, PAS, or Grocott methenamine silver. Branched filaments can be demonstrated in the inflammatory infiltrate. *Nocardia* can also grow in large colonies, forming massive granules (i.e., mycetomas).

Rickettsial Infections

Rickettsial infections are transmitted through several vectors and are caused by obligatory intracellular microorganisms. All rickettsioses are zoonoses, and all rickettsiae are found in arthropods. Humans are accidental hosts that do not maintain these organisms in nature (230). All rickettsioses cause significant clinical disease, and most cause renal involvement, some with mild renal insufficiency and some with acute renal failure and fatal outcomes.

Rocky Mountain Spotted Fever

Rocky Mountain spotted fever, caused by *Rickettsia rickettsii*, is transmitted by bites of ticks. Small mammals function as reservoirs. Patients present with fever, headache, myalgia, nausea, and vomiting, followed by a rash. Exposure to habitats containing ticks is a key epidemiologic factor (230). Disseminated rickettsial infection causes increased vascular permeability, hypotension, and shock with prerenal azotemia. Some patients develop acute renal failure and, on biopsy, show inflammatory infiltration with mononuclear cells and neutrophils, edema, and tubular necrosis. The inflammation is more frequent around vessels and has a predilection for the outer medulla and corticomedullary junction. Figure 24.41 is from a specimen from a patient who died of Rocky Mountain spotted fever and shows rickettsial antigen within endothelial cells. Thrombosis of small capillaries may occur. Although the diagnosis can be made by serologic tests, immunopathologic identification of *Rickettsia* in skin lesions is the only approach that results in a timely diagnosis of acute disease. Renal failure and tubulointerstitial nephritis may resolve or may contribute to death (230).

Mediterranean Spotted Fever

Rickettsia conorii, the etiologic agent of Mediterranean spotted fever, also causes tubulointerstitial nephritis. The infection may manifest with acute renal failure. Shaked et al. reported that 5 of 31 patients with this condition developed renal impairment (231). In three patients, the kidneys showed multifocal perivascular tubulointerstitial nephritis. The inflammatory infiltrate consists of lymphocytes and histiocytes. Features of ATN also can be found and are thought to result from fluid loss owing to vascular leakage and fever.

Scrub Typhus

Scrub typhus, caused by *Rickettsia tsutsugamushi* and transmitted by mites, presents with a primary lesion (i.e., eschar), generalized lymphadenopathy, fever, rash, and myalgia. Renal involvement is infrequent and is comparable to that of other rickettsial infections but milder. Vascular necrosis and thrombosis are rare. Lymphohistiocytic inflammatory infiltrate is vasocentric, predominantly interstitial with associated edema and enlargement of tubular cells. Acute renal failure is rare but does occur (232).

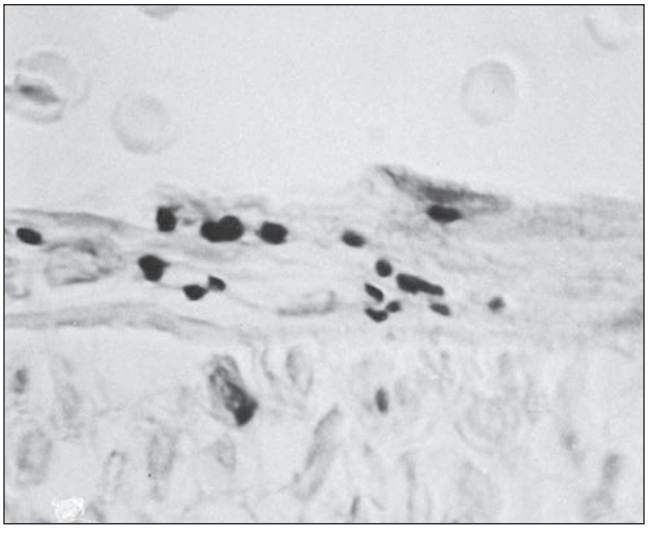


FIGURE 24.41 ***Rickettsia rickettsii* detected with immunoperoxidase using a polyvalent antiserum against *R. rickettsii*.** Dark-staining areas in the endothelium of a small blood vessel of the kidney indicate sites of rickettsiae antigen (×1475). (Courtesy of Dr. Steven Dumler.)

Epidemic Typhus

Epidemic typhus, caused by *Rickettsia prowazekii*, is transmitted by body lice and usually occurs under conditions of crowding, poor hygiene, and poor sanitation. Patients present with fever, myalgia, hypotension, petechiae, jaundice, proteinuria, hematuria, and pyuria (233). The diagnosis can be made by serologic and other tests or by PCR (233). The interstitial mononuclear inflammatory infiltrate is vasocentric and forms typhus nodules. Vasculitis and endothelial cells containing rickettsiae are common. Schumann et al. (234) reported a case of epidemic typhus with fever, renal insufficiency, high titer of antibodies to *R. prowazekii* and *R. mooseri*, and relevant epidemiologic data in a 58-year-old woman. This patient was treated with doxycycline with improvement, but she developed acute renal failure 5 days after treatment was instituted. Her renal biopsy findings, 8 days after antibiotic treatment, demonstrated granulomatous tubulointerstitial nephritis. Continued treatment with doxycycline resulted in improvement of renal function and a decline in the titer of antibodies to *R. prowazekii*. More recently, several case reports and epidemiologic studies show that scrub typhus continuous to be not an infrequent problem for indigenous populations as well as travelers in Asia (235).

Rickettsiae enter cells by receptor-mediated endocytosis through the cholesterol receptor. Once endocytosed, they leave the endocytic vacuole and multiply in the cytosol. Injury to endothelial cells is mediated, at least in part, by superoxide radicals (235). Endothelial cells, smooth muscle cells, or both are infected, resulting in increased vascular permeability, vasculitis, thrombosis, and hemorrhages. Thrombosis appears to result from enhanced endothelial cell tissue factor expression and from release of von Willebrand factor from Weibel-Palade bodies.

Leptospirosis

Several species of *Leptospira* can cause human infections: *L. icterohaemorrhagiae* (i.e., Weil disease), *L. pomona*, *L. bataviae*, *L. shermani*, and *L. grippotyphosa*. Among 50 patients reported by Lecour et al. (236), *L. icterohaemorrhagiae*, *L. canicola*, and *L. grippotyphosa* were responsible for infections in 39, 6, and 2 patients, respectively. Patients present with renal dysfunction or acute renal failure and, in the case of Weil disease, jaundice.

Leptospirosis is a ubiquitous enzootic disease. Animal reservoirs include rodents, skunks, foxes, livestock, ducks, and frogs. The animals demonstrate prolonged urinary shedding of the organism. Humans acquire the microorganism when they are in direct contact with infected tissues or fluids or with contaminated water. Renal involvement is very common, occurring in 44% to 67% of patients (236). Tubulointerstitial nephritis is the main pattern of renal injury in leptospirosis.

In Weil disease, the kidneys are enlarged, edematous, and brownish yellow with petechial hemorrhages. Microscopically, they show ATN, vasculitis, and an interstitial inflammatory infiltrate composed of lymphocytes, monocytes, plasma cells, and neutrophils (237). The outcome of the disease, even if

untreated, is relatively good. However, in elderly patients, the mortality is higher. Antibiotic therapy, particularly penicillin, is effective.

The pathogenesis of the interstitial nephritis may be secondary to the direct infection of the renal parenchyma by the microorganism. There is evidence that the outer membrane protein of leptospira has an important pathogenic role (237). The outer membrane protein activates nuclear factor κB, stimulating inducible nitric oxide, monocyte chemoattractant protein-1, and TNF-α expression (237).

Parasitic Infections

The spectrum of renal diseases associated with parasites has been summarized previously (238). Most glomerular lesions are proliferative, that is, membranoproliferative or mesangioproliferative. However, membranous glomerulopathy, focal segmental glomerulosclerosis (FSGS), and minimal change disease are sometimes seen. Clinically, patients may present with isolated proteinuria or hematuria, nephrotic syndrome, nephritic syndrome, renal insufficiency, or rapidly progressive glomerulonephritis.

Granulomatous tubulointerstitial nephritis caused by parasites may result from their localization in the kidney (e.g., filaria) or from renal localization of their eggs (e.g., schistosomiasis). In malaria, the parasite is found in parasitized red blood cells in the kidney. Tubulointerstitial lesions may reflect complex interactions of various factors, including inflammatory mediators and ischemia (239).

Malarial Infection

Malaria is commonly transmitted by the bite of mosquitoes infected with *Plasmodium*. Less commonly, organ transplantation can be a vehicle for malaria infection (240,241). *Plasmodium falciparum*, *P. malariae*, and more recently, *P. vivax* and *P. ovale* have all been reported to involve the kidney (242). Acute renal failure complicates *P. falciparum* infection in 1% to 4% of patients in endemic areas (243). Interestingly, acute renal failure is much more common in Europeans who do not live in endemic areas. Studies indicate a 25% to 30% incidence of acute renal failure among Europeans infected with malaria (243).

Dialysis has led to considerable improvement in the outcome of patients with malaria-related tubulointerstitial disease. Overall mortality caused by renal failure has decreased; patients who died in a UK referral center succumbed to complications associated with prolonged stay on ICU rather than malaria per se (244). In countries where *P. malariae* (quartan malaria) is endemic, a different spectrum of nephrotic syndrome etiologies is seen. Good accounts of the historic and geographic aspects have been given (245).

Clinical Presentation

Glomerular involvement is common and may result in proteinuria, hematuria, and nephrotic syndrome. Interestingly, only *P. malariae* is associated with nephrotic syndrome. In contrast, *P. falciparum* infection is more commonly associated with acute renal failure. Chronic glomerular disease is classically associated with *P. malariae* infection (quartan malarial nephropathy). *Plasmodium malariae* and *P. falciparum* cause tubulointerstitial injury related to hypotensive or ischemic insult (239,246). Patients may develop hemoglobinuric acute renal failure known as *blackwater fever*, so named because the urine is dark due to large amounts of hemoglobin (247). Among patients with *P. malariae* infection, clinical findings are similar. The nephrotic syndrome occurred in both children and adults. Proteinuria was poorly selective, and steroid responsiveness was generally poor.

Pathologic Findings

In patients with *P. falciparum* infection, renal biopsy may show ATN, interstitial nephritis with predominantly lymphocytes, interstitial edema, hemoglobin casts, tubular hemosiderin, and glomerular mesangial and endothelial proliferation (246). Mesangial deposits of IgM and C3 with or without IgG may be present. The histologic findings of *P. falciparum* infection are apparently reversible (248). Renal biopsy findings in patients with *P. malariae*–associated renal disease vary somewhat from country to country. In the 77 cases reported from Uganda (31 children and 46 adults), 55 showed signs of proliferative glomerulonephritis (249); 3 had an accentuated lobular pattern (MPGN); 11 had focal segmental proliferative disease; 8 had extensive global sclerosis. It was stated that 31 patients had evidence of quartan malaria and that all but one showed proliferative lesions on histology.

Thickening of the glomerular capillary wall with luminal narrowing occurs as the disease progresses. The thickening involves the subendothelial aspects and shows a double-contour or plexiform arrangement of PAS-positive, argyrophilic fibrils (Fig. 24.42). This process, together with mesangial sclerosis, leads to FSGS, eventually becoming more extensive and involving the entire tuft. Cellular proliferative changes are not a feature, except for rare localized mesangial hypercellularity. Tubular atrophy and interstitial chronic inflammation are proportionate to the degree of glomerular involvement.

In reported cases, immunofluorescence studies revealed one of three patterns. The first consisted of coarse, medium-sized granular capillary wall deposits of IgG, IgM, or C3; the second showed very small, fine deposits homogeneously

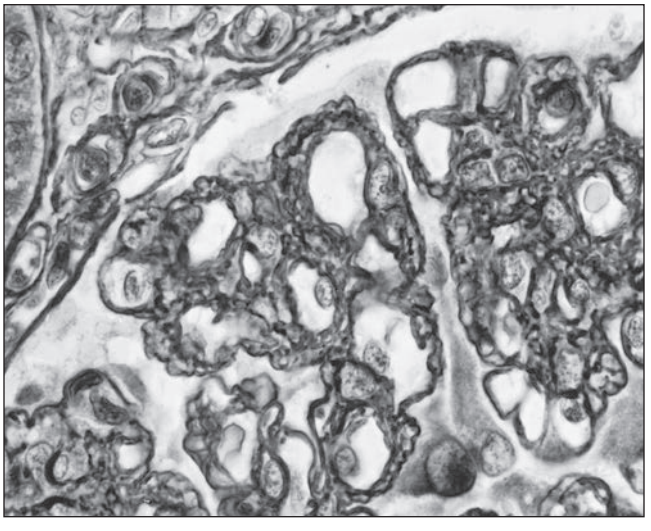


FIGURE 24.42 Double contours and plexiform arrangement of glomerular capillary walls in a patient with malarial nephrosis. (silver impregnation × 1080.) (Courtesy Dr. L. Morel-Maroger.)

distributed along capillary walls with IgG and, in one case of seven, with C3; the third pattern was a mixture of the first two. *Plasmodium malariae* antigen was detected in one of three biopsies tested, but *P. falciparum* antigen was never found.

Ultrastructural analysis showed effacement of foot processes and thickening of the glomerular basement membrane, caused by an increase in subendothelial basement membrane–like material of varying density. Small lacunae in the basement membrane were invariable features. These often contained material comparable in electron density to the basement membrane.

Other histologic findings encountered in the patients with nephrotic syndrome were minimal change disease, MPGN, FSGS, and amyloidosis. It is noteworthy that 7 of 12 patients with these abnormalities had quartan malaria parasitemia.

Renal transplantation may also be a source of malarial infection. Two renal transplant recipients infected with *P. vivax* and *P. ovale* demonstrated fever and renal dysfunction that coincided with parasitemia on days 20 and 8, respectively, after transplantation (240). In the patient infected with *P. vivax*, renal biopsy demonstrated intense tubulointerstitial nephritis with mononuclear cell infiltration. Renal function returned to normal in both patients following antimalarial therapy.

Etiology and Pathogenesis

Given the complexity of disease pathology manifested by *Plasmodium* sp., a variety of pathogenetic factors have been implicated. Acute renal failure is thought to result from microcirculatory blockade by parasitized cells and from nonspecific inflammatory factors (246). Vascular occlusion is caused by endothelial attachment of parasitized red blood cells through a histidine-rich protein. Evidence suggests that quartan malaria can produce nephrotic syndrome and that immunologic injury plays a part. Recent studies have shown that malaria induces a significant decrease on surface complement receptor CR1 expression in the monocyte/macrophage population in mice infected with malaria (250). This results in deficient internalization of immune complexes by monocytes/macrophages. The levels of surface CR1 on peripheral monocytes/macrophages and B cells of malaria-infected patients show a significant decrease compared with control patients suggesting that decrease in CR1 plays an essential role in impaired IC clearance during malaria (250).

Malarial antigen is present in the initial lesion, but progression of the disease does not appear to be caused by a constant supply of malarial antigen. It is suggested that immune complexes may trigger a sequence that could be autoimmune in nature, supported by clinical studies that demonstrated anti-DNA antibodies, among others, in patients with malaria.

Schistosomiasis

Schistosomes are trematodes (flukes) whose intermediate hosts are snails and definitive hosts are humans. There are three major species of schistosomes: *Schistosoma mansoni*, *S. haematobium*, and *S. japonicum*. Infections with *S. mansoni* and *S. haematobium* are associated with significant renal disease. Infection with *S. mansoni* results in glomerulonephritis presenting with proteinuria or nephrotic syndrome. Prospective studies reported evidence of renal disease in 15% of patients with hepatosplenic schistosomiasis in Egypt (251). Infection with *S. haematobium* initially presents with hematuria. Later in the disease course, patients may develop obstructive uropathy (252).

Schistosoma mansoni infection occurs in most sub-Saharan African countries, Egypt, Libya, some Arabian countries, Brazil, and a few other countries in South America (251–254). The adult worms live in the hepatic portal system. Most of the pathologic lesions occur in the liver and spleen. The eggs have a lateral spine and are passed in the feces. In contrast to the lateral spine present on eggs of *S. mansoni*, *S. haematobium* eggs have a terminal spine.

Glomerulonephritis associated with *S. mansoni* infection is geographically variable, and histologic patterns have recently changed (253,254). Schistosomal glomerulopathy is typically referring to MPGN, which was once the most prevalent pathology. However, currently, MPGN has declined to about 50% of all schistosomiasis-associated glomerular pathology at least in Brazil (253). Early in the disease course, mesangial IgM and C3 are identified by immunofluorescence. All species of schistosomes may present in this fashion. Immunofluorescence shows endocapillary granular C3 deposits. FSGS is the second most common entity. *Schistosoma mansoni* is the most common offending agent when MPGN and FSGS are present. Immunofluorescence may show mesangial IgG and C3 or granular deposits of IgM and C3 in the mesangium and capillary walls. Later in the disease, glomerular IgA is present. Membranous glomerulonephritis, exudative glomerulonephritis with endocapillary neutrophils, and amyloidosis are described less often.

Barsoum (251) has emphasized the prominence of mesangial IgA deposits in the MPGN and FSGS forms of glomerular involvement. Since these lesions are associated with significant hepatic pathology, it is thought that liver disease contributes to the pathogenesis of the glomerular lesion. Serum IgA antigliadin, serum anti-DNA antibodies, and glomerular IgA deposits are markers of significant renal involvement in patients with hepatosplenic schistosomiasis.

Three types of interstitial inflammation occur in schistosomal nephropathy. The first is a granulomatous reaction to eggs in the kidney (256). Eggshells can be readily identified in tissues. *Schistosoma mansoni* and *S. japonicum* eggs are acid-fast, while *S. haematobium* eggs are not. The second more common response is mononuclear cell inflammation and interstitial fibrosis associated with obstructive uropathy (252). The third is interstitial inflammation and fibrosis accompanying schistosomal glomerulopathy. Classically, urinary tract disease is a feature of *S. haematobium* infection. Squamous cell carcinoma of the urinary bladder is typically associated with infection by this parasite (257–259).

Studies have suggested a cause-and-effect relationship between *S. mansoni* infection and glomerular lesions. It was shown that (a) schistosomal antigen was present in the glomeruli (255), (b) antibody reactive with adult worm antigen was eluted from kidneys with glomerular lesion, (c) circulating immune complexes were detected in patients with *S. mansoni* infection, and (d) glomerular lesions were produced in a variety of animals by injection of *S. mansoni* (253). These findings suggest an immune complex pathogenesis for the glomerular lesions. The dominance of IgA in the glomerular deposits of the chronic and progressive forms of disease suggested that altered hepatic clearance of IgA aggregates or immune complexes from the circulation can contribute to glomerular

immune injury in the absence of active infection. Factors suggested for FSGS associated with schistosomiasis implicate Th2 immune response. It appears that certain polymorphisms in the IL-13 gene increase susceptibility to schistosomiasis by increasing cytokine production, which subsequently cause podocyte injury and secondary FSGS (260).

Clinical Course, Prognosis, and Therapy

The overall course of the membranoproliferative and focal segmental glomerular sclerosis variants are comparable to the idiopathic forms. At the end of 5 years of follow-up of 25 patients with *S. mansoni*–related MPGN, renal function was normal in 7, renal insufficiency was present in 11, and end-stage renal disease was seen in 7. Of 25 patients with *S. mansoni*–related focal and segmental glomerular sclerosis followed for 5 years, 17 had normal renal function, 4 had renal insufficiency, and 4 had end-stage renal disease. A prospective study of 12 patients with hepatosplenic schistosomiasis, nephrotic syndrome, and FSGS followed patients for more than 12 years (125 ± 9.1 months) (261). Four patients were in complete remission with normal renal function, and one was in relapse; seven patients had persistent proteinuria, with progression to renal insufficiency in six and to end-stage renal disease in three. End-stage kidney disease is common in such cases. Response to treatment with antischistosomal drugs is poor. The search for new drugs to combat resistance to known agents has led to experimental exploitation of tyrosine kinase receptor inhibitors. Taking advantage of the similarity between the catalytic domains of *S. mansoni* insulin receptors (SmIR1 and SmIR2) and venus kinase receptors (SmVKR1 and SmVKR2), several compounds were synthesized that lead to death of schistosomal eggs and adult worms at low concentrations (262).

Filariasis

The usual clinical presentation of filarial-associated glomerulonephritis is proteinuria, often nephrotic range, with hypertension in 75% and severe impairment of renal function in 48% of patients. Glomerulonephritis is a well-known complication of filarial infections, including *Wuchereria bancrofti, Onchocerca volvulus*, and *Loa loa* (263). A clear association is difficult to establish because of frequent coinfections (hepatitis B and malaria).

Renal biopsy findings are variable. MPGN, membranous glomerulonephritis, and minimal change disease and FSGS are reported in patients with filariasis (263,264). Microfilariae may cause severe tubulointerstitial inflammation with lymphocytes, plasma cells, and eosinophils, with or without granulomas. The transplant population is not spared in endemic areas. A rare case of filariasis mimicking lymphocele in a kidney transplant recipient is reported (265).

Microsporidiosis

Microsporidia, which belong to the genus *Encephalitozoon*, are obligate intracellular protozoa. Five microsporidia are reported to infect patients with AIDS—*E. bieneusi, S. intestinalis*, *E. hellem*, *Pleistophora,* and *E. cuniculi*. *E. cuniculi*, the prototypic species of this genus, targets the central nervous system and kidneys with involvement of macrophages, epithelium, and endothelium. In hematoxylin and eosin–stained sections, the oval to slightly elongated, 1- to 2-μm spores have a blue staining outline and either a central blue band or a dense blue pole opposite a clear polar vacuole. Spore detection is enhanced by Gram staining, polarization, and fluorescence chitin stains (266). Morphologic speciation is imprecise and requires antigenic and molecular studies, including PCR.

Tubulointerstitial nephritis can occur with disseminated *S. intestinalis*. Although the glomeruli contained occasional microsporidial spores, infection was concentrated in the distal nephron, especially in the medulla. Parasitophorous vacuoles expanded the cytoplasm of attached and sloughed tubular epithelial cells. Tubular lumina also contained spore-laden macrophages, cellular debris, and free spores. Progressive destruction of tubules led to microabscess-like foci rich in macrophages, debris, spores, and scattered neutrophils. Some infected pyramids were necrotic. The most recent microsporidiosis cases were reported in kidney transplant receipts (266).

Amoebiasis

Amoebiasis is a protozoal infection caused by *Entamoeba histolytica*. Renal involvement is very rare. In invasive infections, the kidney is the fifth most common site of abscess localization (267). Trophozoites can be found within foci of liquefactive necrosis and abscess formation.

Echinococcal Infection (Hydatid Disease)

Cystic hydatid disease is caused by the larval form of *Echinococcus granulosus*. It is endemic in parts of Africa, Latin America, the Mediterranean, the southeast of the United States, Iran, and Turkey. Renal involvement comprises 2% to 4% of all cases (268). The kidney is usually involved as part of disseminated disease. Echinococcal larvae may reach the kidneys through the bloodstream, lymphatics, or by direct invasion. Isolated renal echinococcosis is extremely rare. The most common symptoms are palpable mass, flank pain, hematuria, malaise, and fever. Hydatiduria is pathognomonic. Patients pass typical grape-like material in the urine. This occurs in 5% to 25% of cases. Treatment is mainly surgical. However, partial nephrectomy and percutaneous drainage carry the risk of dissemination and fatal anaphylactic reaction.

Most reports of renal involvement by hydatid disease concern renal cysts. A few cases of glomerular lesions have been reported. Microscopically, minimal change disease, membranous glomerulopathy, and MPGN have been reported (269). In most cases, the glomerular lesions are reversed by treating the infection. In one patient with membranous nephropathy, echinococcal antigens and antibodies were eluted from renal tissue, supporting an immune complex–mediated pathogenesis.

Leishmania

Leishmaniasis is a parasitic disease characteristically transmitted to humans by the bite of the sandfly. Approximately 20 species of Leishmania may be transmitted to humans via approximately 30 species of sandflies (270). Occasionally, the disease may be transmitted through organ transplantation. Patients infected with the HIV are particularly susceptible given their immunosuppressed status (271,272). In addition to direct infection, leishmaniasis may result from reactivation of latent disease in immunosuppressed patients (270). Leishmaniasis causes mucocutaneous and visceral syndromes. Visceral leishmaniasis, also known as kala-azar, is the second leading cause of mortality after malaria in the endemic regions of Southeast Asia, East Africa, South America, and the Mediterranean (270).

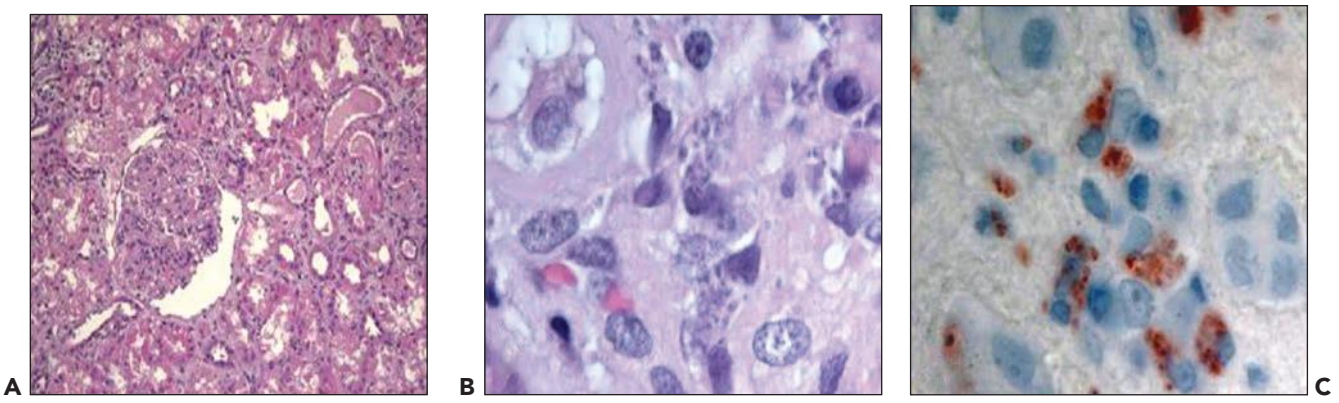


FIGURE 24.43 Leishmania. A: Membranoproliferative glomerulonephritis is seen on H&E (×10). **B:** Organisms are clearly visible when viewed under high magnification. (H&E × 100.) **C:** Immunohistochemical staining for Leishmania antigen confirms the diagnosis. (Courtesy of Kerstin Amann, Erlangen, Germany.)

Renal involvement by *Leishmania* spp. may manifest with acute renal failure, renal amyloidosis, glomerulonephritis, or nephrotic syndrome (271). Many studies have been performed on dogs since they are the natural animal reservoir in the Mediterranean. Dogs may show MPGN due to infection with *Leishmania* spp. (271). Case reports of human visceral leishmaniasis have also described MPGN pathology (Fig. 24.43) (271). Although renal involvement is rare in Leishmania infection, it should be considered in patients from endemic areas, particularly those who are immunosuppressed.

REFLUX NEPHROPATHY

Introduction and Definitions

Reflux nephropathy is defined as renal scarring secondary to retrograde flow of urine from the lower to the upper urinary tract without physical obstruction. Reflux nephropathy is distinguished from pyelonephritis, which entails inflammation of the renal pelvis secondary to infection irrespective of reflux, and from kidney hydronephrosis manifested by renal pelvis and calyceal dilatation secondary to physical or functional obstruction (obstructive nephropathy). Reflux mainly refers to VUR due to an incompetent ureterovesical valve situated at the site of entry of the ureter into the bladder known as ureterovesical junction (VUJ). The normal ureter enters the urinary bladder obliquely and extends into the submucosa at a length that in adults averages 1.3 cm and in children 0.5cm (273). Physiologic VUJ function requires coordination of multiple factors, including correct insertion of the ureter into the bladder, an adequately long intramural ureteral segment, appropriate width of the ureteric opening, and muscle contraction at the trigone. Galen and Leonardo da Vinci were the first to describe the importance of the UVJ as a one-way valve that prevents backflow of urine (cited in (274,275)). In the last 100 years, an explosion of experimental work in humans and animals has shed new light in the pathogenesis of reflux, particularly its genetic basis. A brief historic perspective for reflux from Galen to mouse models is shown in Table 24.4. Urine reflux can be congenital (primary), secondary (associated with other congenital urinary anomalies), acquired, and/or familial. Primary reflux, unilateral or bilateral, is due to incompetent VUJ at the point of the ureteral orifice. Secondary VUR in children is due to congenital anomalies associated with incompetent VUJ, for example, duplicate ureters, crossed renal ectopia, or pelvic (horseshoe) kidney (276). In adults, reflux is often acquired, due to neurogenic bladder and repeated UTIs causing VUJ inflammation and edema. Occasionally, reflux occurs in pregnancy and in the transplanted kidney. Reversal of urine flow into the ureter, renal pelvis, and calyces in the mature kidney may damage the renal parenchyma both by increased fluid pressure and/or contamination with pathogenic organisms present in the bladder urine. The combination of VUR and renal scarring were previously known as atrophic pyelonephritis, a term that erroneously considered all scarred kidneys in VUR to have had infections. Atrophic pyelonephritis is now replaced by the term reflux nephropathy (RN). Radiologists and urologists tend to use the anatomic term VUR focusing on the underlying structural abnormality that may be treated with surgery. Radiologists define RN as simply renal scars, irrespective of pathology. However, kidney specimens have a broad spectrum of gross and microscopic pathologic findings that differ based on whether VUR is congenital or acquired, as described below.

Incidence and Clinical Presentation

Incidence of VUR is estimated to about 1%. However, recent studies suggest that prevalence of VUR depends on the indications for testing (277,278). For example, 1/3 of girls evaluated for UTIs are diagnosed with VUR, and 10% of boys with antenatal hydronephrosis have VUR. VUR is much less common in black compared to white children. Siblings of affected children are at high risk with an incidence that varies from 4.7% to over 50% (277–279). The great majority of VUR is mild and resolves within 2 years. High-grade reflux often exists with other urologic anomalies. For example, in a retrospective study of 1765 consecutive cases in Ireland, 229 (13%) of children of those diagnosed with high reflux duplex ureters were found in 64.6%; 29 children had bladder diverticula, 12 had a solitary kidney, 13 had a ureterocele, 11 had hypospadias, 9 had ureteropelvic junction (UPJ) obstruction, 3 had malrotated kidney, 2 had horseshoe kidney, and 1 had crossed ectopy (276).

TABLE 24.4 Reflux Nephropathy: Historical Perspective

Year	Investigator	Advance
100 AD	Galen of Pergamon and Asclepiades	Discovered they could not induce reflux in live or dead animals suggesting that VUJ is a one-way valve
1487–1513	Leonardo da Vinci	Reflux is accompanied by scarred kidneys.
1812	Charles Bell	Oblique course of the ureter
1893	Pozzi	Reflux in humans
1903	Samson and Young	Normal obliqueness of the ureter prevents reflux.
1913	Legueu and Papin	Hydronephrosis and hydroureter with urine reflux caused by a widely patent ureteral orifice
1914	Kretschmer	Familial reflux
1929	Gruber	Incidence of VUR varied based on the length of the intravesical ureter and muscularity of the detrusor muscle.
1950	Hutch	Pathophysiology of reflux in paraplegic patients and use of VCUG in patients with unexplained hydronephrosis
1960	Hodson	Radiologic diagnosis of VUR
1965	Tanagho	Incision in the trigone distal to the ureteral in dogs induced reflux.
1975	Ransley and Risdon	Resection of the roof of the subcostal ureteral tunnel in piglets induced reflux.
1985		The International Reflux Grading System established
2000s		Mouse models and genetic studies

From Refs. (273–275).

Congenital reflux in infants and young children may be asymptomatic or present with nonspecific lower urinary tract symptoms, including dysuria, flank pain and tenderness or fever, hypertension, or proteinuria. End-stage renal disease (ESRD) occurs in about 15% to 25% of patients (277). Repeat UTIs associated with VUR carry a significantly higher risk for renal scarring compared to UTI without VUR. Several studies confirm this risk. For example, a systematic review of 1533 articles in the published literature found that 57% of children with a first UTI had radiologic evidence of acute pyelonephritis and 15% had evidence of renal scarring based on a renal scan 5 to 24 months later (278). Children with grade III VUR or higher were more likely to develop kidney scars than children with lower grades of VUR. Such studies emphasize that sole focus on VUR in deciding patient management is not enough to assess the risk of permanent renal damage and suggest that UTI be taken into account (279). However, the issue is currently controversial, and there is no agreement as to how to manage UTIs in reflux (see under management) (280,281).

The differential diagnosis of reflux includes common nonanatomic but functional disorder of the lower urinary tract. This category is collectively called dysfunctional elimination syndrome (DES) to include terms such as vesical sphincter dyssynergia, nonneurogenic and neurogenic bladder dysfunction, uninhibited pediatric bladder, and irritable bladder syndrome. Both boys and girls are affected. According to the international reflux study, DES accounts for as many as 18% of children with reflux (282).

Radiologic Evaluation

Diagnosis of VUR is achieved with ultrasound and voiding cystourethrogram (VCUG). Ultrasound is safe, noninvasive, and therefore, the most frequent in-office test for patient follow-up or initial assessment. However, convenient and free of ionizing radiation false results are not infrequent with ultrasound, particularly in low-grade reflux. In contrast, VCUG is reliable and the standard reference test for children. VCUG utilizes fluoroscopy to delineate the bladder outline, bladder neck, and the urethral anatomy and provides a good estimate of the bladder capacity. During voiding, the bladder is filled with a radiocontrast agent through a urethral catheter and using fluoroscopy the distribution of the dye is observed (277,279). Retrograde filling of the upper urinary tract is diagnostic of reflux (Fig. 24.44). The severity (grade) is essentially based on the degree of calyceal dilatation.

Radionuclide cystography (scan) with contrast media such as technetium-99m-mercapto-acetyl-tri-glycine [99mTc-MAG3] is an enhancing test for children who require evaluation for renal scarring. Involvement of the upper urinary tract is evaluated by excretory urography (IVP) and is particularly suited for children with febrile UTIs. One or more scars defined as focal thinning of the renal cortex often overlying a dilated calyx may be identified depending on severity of reflux. A radiographically small atrophic kidney represents end-stage disease.

Grading of Reflux

Reflux is classified in five grades (I to V). Grade I indicates reflux into the ureter only without dilatation. Grade II indicates reflux into the ureter and the renal pelvis and calyces and grade III, reflux into the ureter and pelvis with mild to moderate tortuosity of both. Grade IV is reflux grade III plus complete obliteration of the calyceal fornices, and grade V is reflux into the ureter, pelvis, and calyces with tortuosity of the ureter and gross dilation of the pelvis and calyces with visible papillary impressions. High-grade reflux often referred to as the "back-pressure type" is most frequently the type associated with cortical scars (Fig. 24.45).

Gross Pathology and Light Microscopy

Only severe cases of congenital VUR are examined by pathologists, typically at fetal autopsy. Dilated ureters connect to normal appearing or grossly malformed kidneys. In bilateral

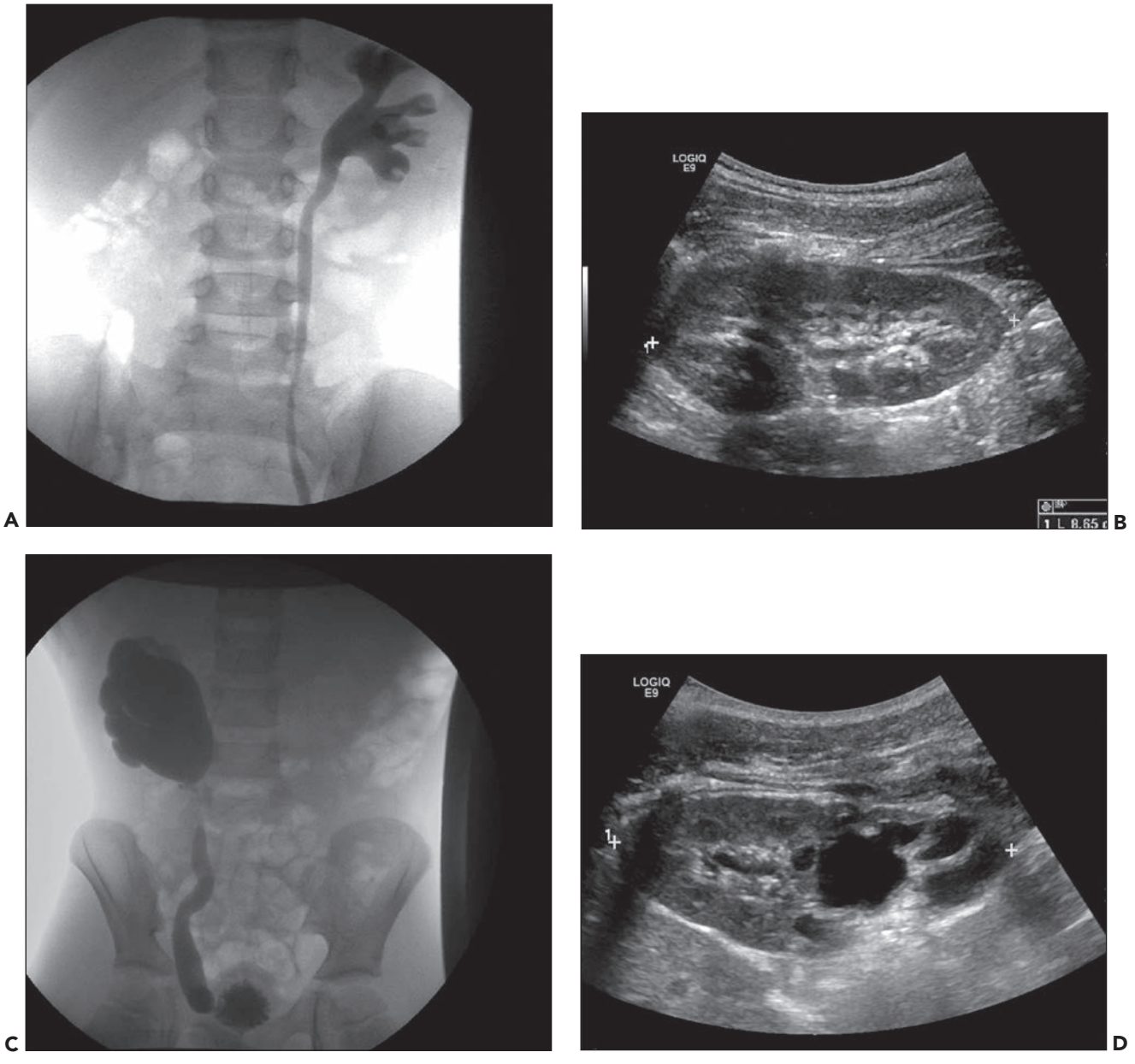


FIGURE 24.44 Low- and high-grade reflux viewed by VUCG. A: VUR in left kidney of a 5-year-old girl with history of UTI, wetting, and soiling shows retrograde filling of the left ureter and renal pelvis, but no dilatation (hydronephrosis) characteristic of low-grade (II) reflux. **B:** Ultrasound shows normal kidney parenchyma. Even with repeated UTIs, this young girl has very little chance of scarring her kidneys. **C:** High-grade VUR and right kidney hydronephrosis from a 5-year-old boy with prenatally diagnosed hydronephrosis (grades III and IV). He has never had a UTI. **D:** Ultrasound shows quite thick and robust kidney. He should do well with time and may never need surgery. (Courtesy of George Steinhardt, Grand Rapids, MI.)

VUR, ureters are dilated often to their full length. An example of bilateral VUR in a male fetus is shown in Figure 24.46. Kidneys are normally lobulated with mild hydronephrosis. Urinary bladder is dilated. Upon dissection, there was no anatomic obstruction found. Unilateral reflux is often associated with a grossly normal contralateral kidney, as in the case shown in Figure 24.47. In other cases, ureters may be tortuous, may have thick muscular wall or atretic ends, and may be duplicated connecting to grossly malformed kidneys (Fig. 24.48). In most specimens, kidneys are smaller than normal, segmentally or diffusely scarred, or cystic. Grossly malformed kidneys or atrophic small kidneys are likely dysplastic histologically (Figs. 24.48 and 24.49). Dysplastic kidneys typically consist of disorganized renal parenchyma with immature glomeruli and collecting ducts, the latter encircled in smooth muscle whorls known as fibromuscular collars. Islands of cartilage are found in about 1/3 of dysplastic kidneys. Sometimes, renal dysplasia is segmental, affecting only part of the kidney; an extra duplicate ureter may be the culprit (Fig. 24.50). An alternative pattern of RN consists of

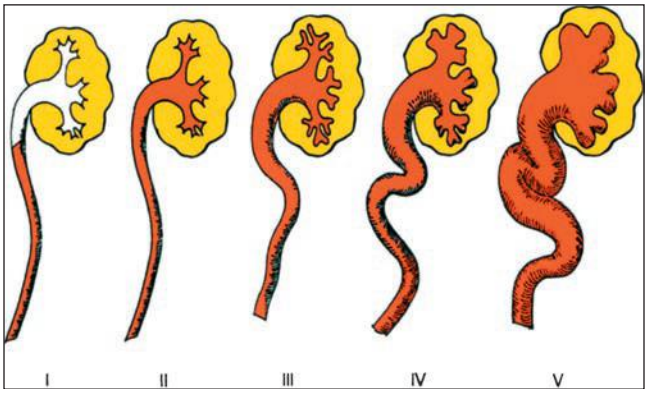


FIGURE 24.45 Grades of VUR. (Modified from Medical versus surgical treatment of primary vesicoureteral reflux: a prospective international reflux study in children. *J Urol* 1981;125:277–283.)

renal cortical scars sharply demarcated from adjacent normal parenchyma and absence of cysts (Fig. 24.51). Scars contain sclerotic glomeruli, atrophic or dilated tubules, and interstitial chronic inflammatory cells but no dysplastic nephrons (Fig. 24.52). These features are similar to obstructive nephropathy. RN scars radiologically may look very similar to renal dysplasia; therefore, the term renal scarring is used indiscriminately by urologists and radiologists to describe both. Bladder outlet obstruction, valves in the posterior urethra, bladder diverticula, ureteroceles (prolapse of the terminal part of the ureter into the bladder appearing as paraureteric saccule), and duplicate ureters are diagnosed radiologically. Of note, the majority of children with VUR have normal kidneys, particularly those that show early resolution of reflux (283,284). Not infrequently, VUR and ureter duplication in children are not isolated findings but part of malformation syndromes (Fig. 24.53), in contrast to adults in whom unilateral ureter duplication may be of no consequence, found incidentally, and with normal kidney function (Fig. 24.54).

FIGURE 24.46 Bilateral vesicoureteral reflux: an 18-week-old male fetus with dilation of ureters and mild hydronephrosis. Kidneys are normally lobulated with no evidence of dysplasia.

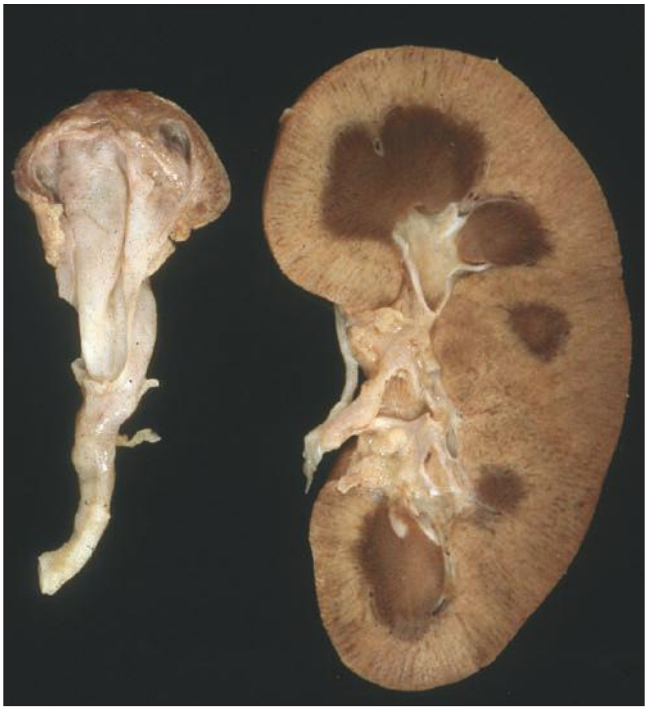


FIGURE 24.47 Unilateral reflux: an 8-month-old girl with hypoplastic left kidney and ipsilateral reflux due to stenotic posterior urethral valve. Contralateral kidney is grossly normal with no evidence of hydronephrosis.

The tissue that composes the vesicoureteral valve is very rarely submitted for routine pathologic evaluation. The unique study by Becu et al. of 27 patients with documented VUR prior to surgery had resected VUJ valves serially sectioned and examined histologically. Fifteen of 27 valves were found to have either a single or a double *flap*. The muscle of the unobstructed segment of ureter distal to the valve appeared normal, in contrast to a hypertrophic muscle of the obstructed proximal segment (285). While this is an informative study, concern was raised about resection artifacts, and this study advocates that currently evaluation of the anatomy of the vesicoureteral valve is best done radiologically. Congenital malimplantation, lateral ectopias of the ureteral orifice, short or duplicate ureters, and abnormal shape and diameter of the ureteral orifice may be so identified along with various grades of reflux. In about 4% of VUR, the ureteral orifice appears normal.

In summary, the most common pathologic findings seen in kidneys with severe VUR are (a) ureter abnormalities, (b) renal dysplasia, (c) renal cortical atrophy without dysplasia, and (d) chronic inflammation and glomerulosclerosis, which are features overlapping with obstructive nephropathy.

As mentioned above, the allograft kidney may be complicated by reflux, and reflux may be complicated by glomerulonephritis. Additional clinically distinct types of reflux include sterile reflux and reflux in pregnant women. Segmental cortical atrophy when associated with hypertension is known as Ask-Upmark kidney. Finally, the category of hereditary reflux is currently an evolving topic and is discussed separately later.

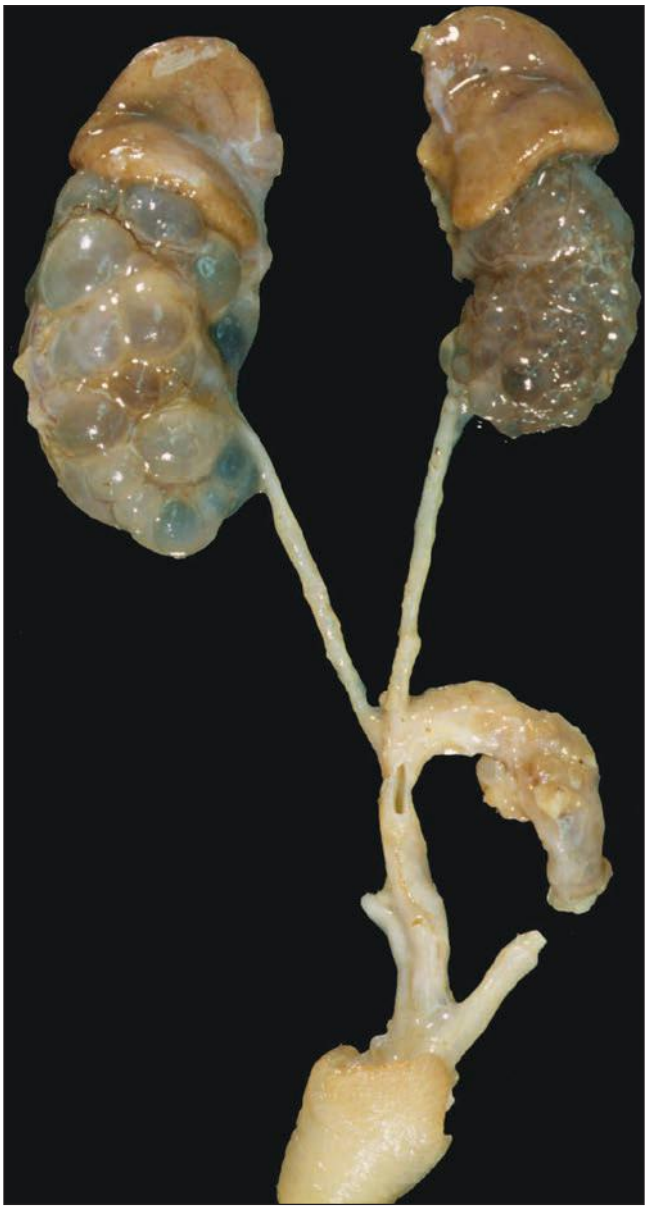


FIGURE 24.48 Stillborn male infant at 21 weeks' gestation born to a 34-year-old mother G7P0. Pregnancy was complicated by anhydramnios secondary to hypoplastic urinary bladder and bilateral multicystic dysplasia. Shown are small multicystic kidneys with ectopic ureters terminating blindly in rudimentary urinary bladder. Maternal history of multiple previous spontaneous abortions raises the question of a genetic cause for the renal anomalies, but there are no genetic studies on record.

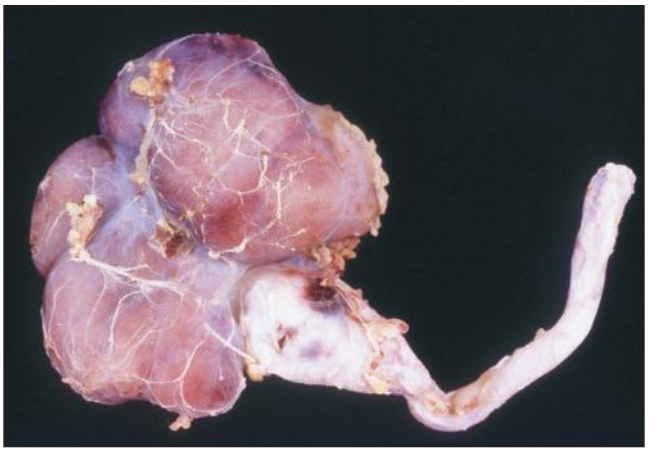


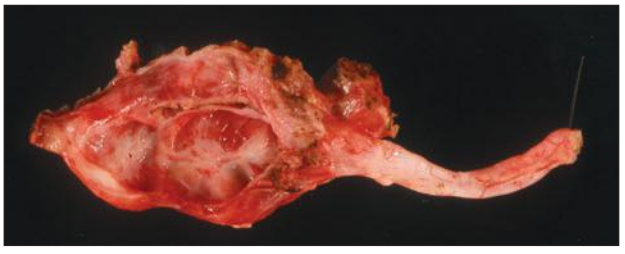
FIGURE 24.49 A six-year-old boy with left kidney hydronephrosis and grade IV reflux with <5% function on scan. Resected left kidney shows scarred cortex and dilated pelvis. Notably, the right kidney also had persistent grades II and III reflux for which right ureter reimplantation was performed during left nephrectomy.

Distinct Types of Reflux

Ask-Upmark Kidney

In 1929, Eric Ask-Upmark first described a renal abnormality characterized by segmental atrophy in six patients who presented with malignant hypertension. Eighty years later, there are more than 200 cases reported in the literature. Ask-Upmark kidney may be unilaterally or bilaterally found both in children and adults, male and female with preponderance for women. The terms segmental hypoplasia and aglomerular segmental hypoplasia have also been used (286–289). Grossly, the affected kidney is reduced in size with one or more sharply separated hypoplastic cortical segments over dilated calyces (Figs. 24.55 and 24.56). Histologically, the thin segment contains atrophic tubules, thick vessels, and no glomeruli (see Fig. 24.56B). Absence of glomeruli is characteristic and contrasts with sclerotic glomeruli seen in other types of cortical atrophy. Severe hypertension is seen in most children and 60% of adults and may be the presenting symptom. Other symptoms include recurrent UTI, proteinuria, and decreased renal function. Ask-Upmark kidney was originally described as a congenital anomaly, but most investigators now consider segmental atrophy to be secondary to parenchymal pressure due to reflux of urine and infection. Others have shown that the atrophic segment is irrigated by a small renal artery branch, and therefore, the lesion is attributed to a developmental defect of the renal vasculature (289). The debate on whether the kidney "scar" is due to urine backflow and superimposed inflammation or a vascular anomaly started with early investigators who argued that maldevelopment of the kidney was unlikely to be due to inflammatory destruction and postulated an ischemic process. Subsequent studies demonstrated that some patients with Ask-Upmark kidney have renin-dependent hypertension substantiating the ischemic hypothesis. In fact, many of the recent reports demonstrate segmental renal artery stenosis, which occurs either as an isolated renal finding or with extrarenal vascular aneurysms (289). Fibromuscular dysplasia associated with Ask-Upmark kidney was also reported (290). The origin and role of renin in this form of hypertension is still under investigation. Some have suggested that the hypoplastic areas may not release renin because of the absence of glomeruli and juxtaglomerular cells and that increased plasma renin may be derived by adjacent tissue. Nephrectomy typically cures hypertension.

The natural history of segmental hypoplasia in Ask-Upmark kidney is little known. In a study of nine children who initially had normal functioning kidneys, scars were



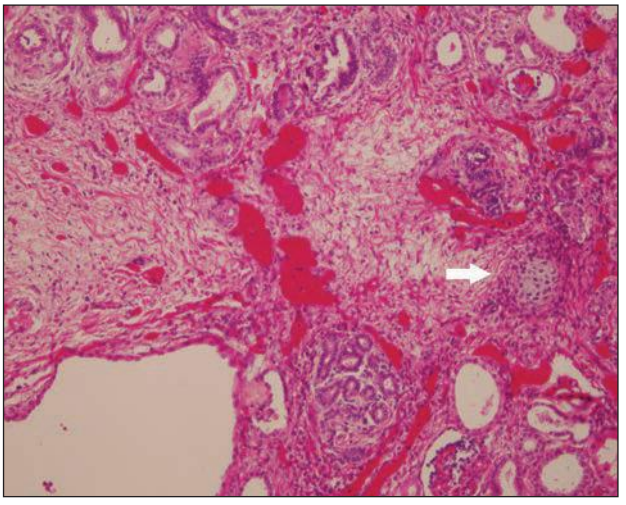


FIGURE 24.50 **A:** Kidney from an 8-month-old girl with left kidney double ureter draining the upper pole. Segmental resection was performed. The kidney in this segment was histologically dysplastic. **B:** Section shows disorganized renal parenchyma with loss of nephrons, primitive tubules, and characteristic focal cartilage (*arrow*) characteristic of renal dysplasia. (H&E × 200.)

evaluated radiographically and histologically (290). Mean time from discovery of reflux to renal scar was 6.1 years, and the onset of hypertension in six patients was 7.8 years. Resected kidneys had lobar atrophy, atrophic tubules, and segmental loss of nephrons. These findings reveal that renal scarring in Ask-Upmark kidney is a progressive as opposed to a static lesion, despite correction of the reflux or prevention of UTI, suggesting optimization of clinical management and timing for surgical intervention. Contralateral or extrarenal vascular anomalies should be ruled out.

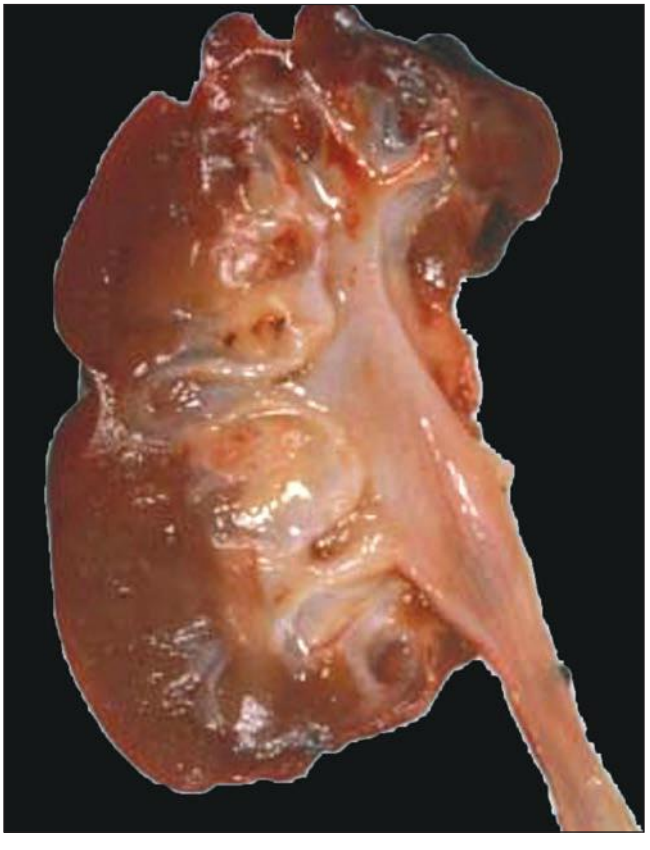


FIGURE 24.51 Segmental cortical scars and dilated pelvis from young child with reflux but no evidence of cysts or renal dysplasia. (Courtesy of Steven Bonsib, Nephropath, Little Rock, Arkansas.)

Hereditary Reflux

VUR is a congenital urinary track anomaly with high frequency of reflux in siblings; twin concordance and parent-child transmission provide strong evidence that VUR is at least in part a heritable disease. Autosomal dominant, autosomal recessive, X-Linked, and polygenetic inheritance are reported (291,292). Four different strategies are employed to search for genes involved in human VUR: (a) screening for mutations in candidate genes

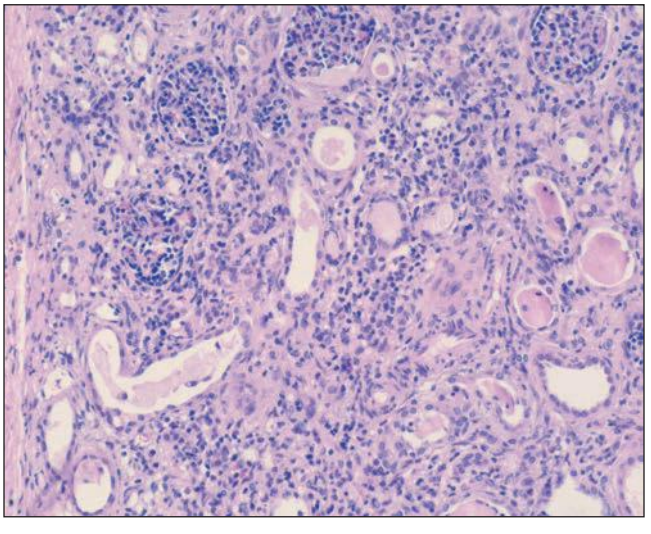


FIGURE 24.52 Reflux nephropathy shows interstitial inflammation and dilated tubules; kidney was resected for severe reflux secondary to posterior urethral valves. (H&E; ×200.)

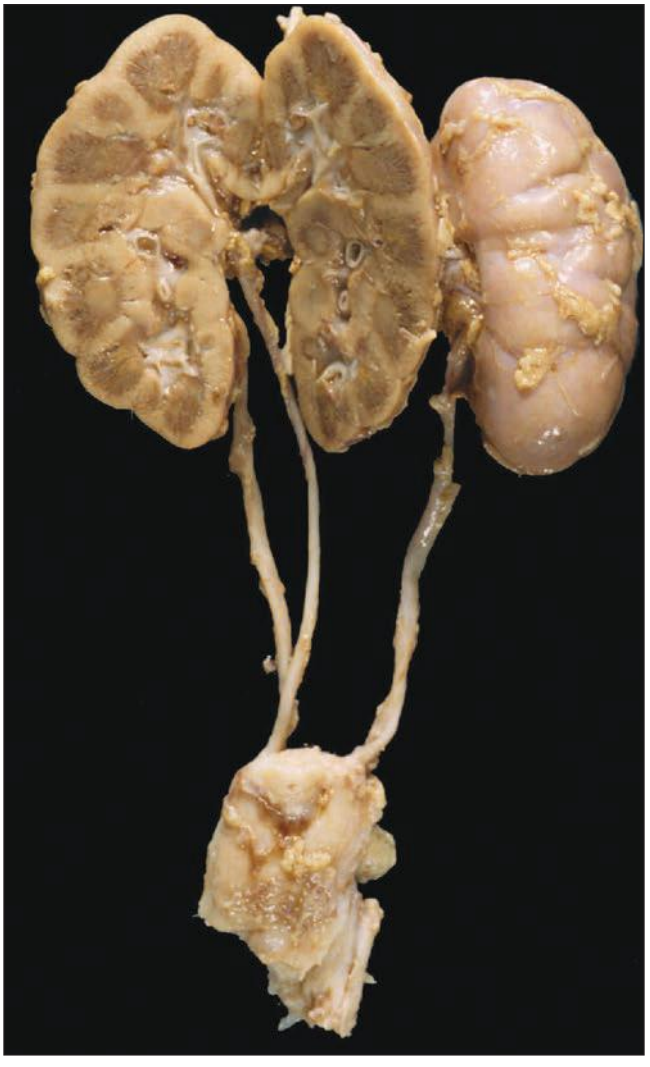


FIGURE 24.53 A 3-month-old girl with Williams syndrome (q11.23 chromosome 7 deletion with loss of about 25 genes in this region associated with supraclavicular aortic and pulmonary stenosis). Left kidney has duplicate ureter (also duplicate renal artery). Renal and urinary tract anomalies are found in 20% patients with Williams syndrome.

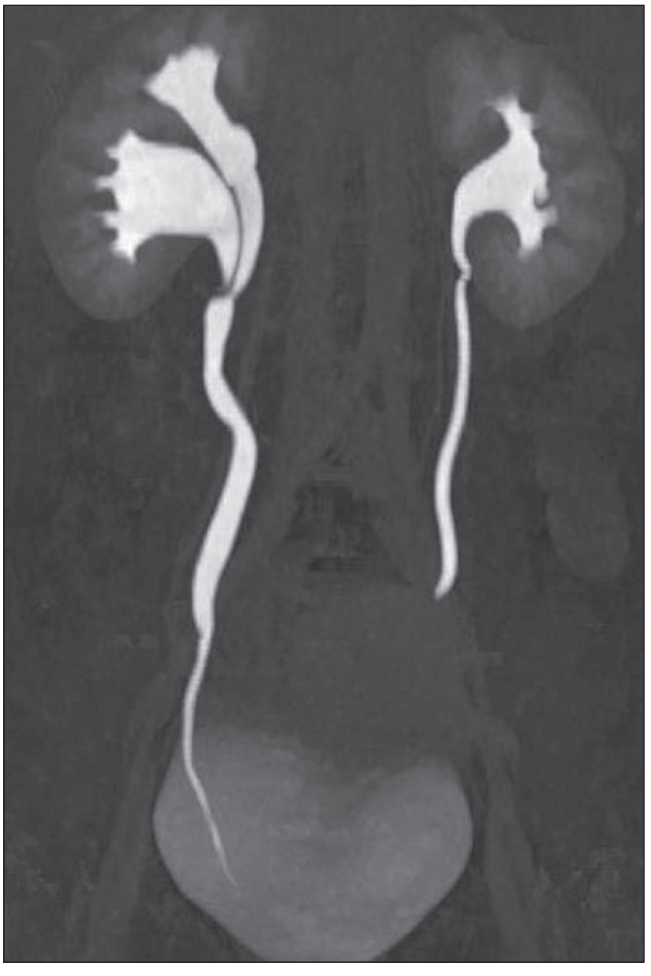


FIGURE 24.54 A 40-year-old woman with history of hematuria has unequal size kidneys with right kidney larger than left and right duplicate ureter but intact-appearing renal parenchyma (retrograde ureterogram).

known from mouse VUR models, (b) genetically characterize chromosomal abnormalities found in patients with VUR to identify new genes, (c) genes known to cause multiple organ malformations in syndromic VUR in patients with nonsyndromic VUR, and (d) whole genome mapping (274,291). With such approaches, more than a dozen genes and additional 10 candidate loci are reported. A short list of genes implicated in human VUR is shown in Table 24.5. The multiplicity of genes and gene mutations within the same gene in humans, contradicting data from different studies and coexistent renal anomalies with VUR, present a confusing literature particularly because mouse and human studies are often discussed together. For each gene, only a small number of patients harbor a specific mutation; therefore, it is anticipated that additional new genes and gene mutations will be detected in humans and the list will be getting longer as individual families are tested.

Feather et al. studied seven European families and found a dominant mode of inheritance with more women than men affected. VUR in these families mapped on chromosome 1, but specific mutations were not identified (293). No mutations in 6p or 10q where PAX2 is located were found in these families either. PAX2 mutations cause the renal-coloboma syndrome (optic nerve coloboma, renal anomalies, and VUR), and therefore, PAX2 was thought a good candidate gene for VUR. However, linkage analysis and mutation scanning revealed limited results so far (294,295). In the spectrum of renal tract malformations, VUR has been linked to gene mutations of the angiotensin II receptor type 2 (AGTR2) located on Xq, which in knockout mice induces VUR. A-1332G transition in the AGTR2 receptor gene was found in primary obstructive megaureter and pelviureteric junction obstruction but not in humans with primary VUR (296,297). The authors proposed that while AGTR2 is crucial for the normal development of the ureter, it does not appear to contribute to the processes that lead to VUR, which they consider primarily an abnormality in the bladder trigone. However, an Italian study found

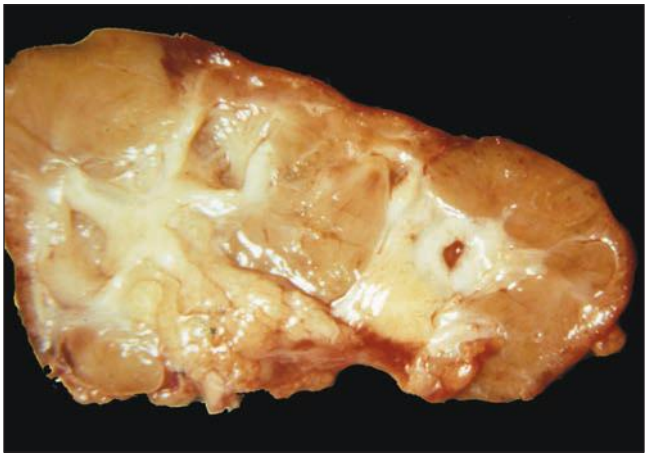


FIGURE 24.55 **Ask-Upmark kidney from a young girl.** Renal cortex is thin above dilated pelvis.

TABLE 24.5 **Partial list of genes involved in human VUR**

Gene	Mutations in humans
Pax2	Optic nerve colobomas and familial renal anomalies, 30% of which have VUR.
AGTR2	VUR and CAKUT
ACE	VUR alone
SOX17	VUR
ROBO2/SLIT2	High-grade VUR + other renal anomalies
RET/GDNF	Primary reflux, duplicate ureters, megaureter, and CAKUT
BMP4	CAKUT including VUR
Eya1	VUR, duplex ureters, CAKUT, renal agenesis
FOXC1	CAKUT with VUR
TGF-β	High-grade VUR

From Refs. (292–326).

AGTR2 polymorphisms in about 50% of their VUR patients and suggested that the discrepancy with previous studies may be due to genetic and ethnic backgrounds (298). Similarly, screening of families for uroplakin III, a gene associated with abnormal ureteral orifice in mice, revealed no mutations in humans (299,300). It was concluded that such mutations may be lethal in humans; however, the authors did not rule out mutations in regulatory elements of the gene that may affect uroplakin expression and function. BMP4 and FOXC1 genes are two other genes that in mice are shown to participate in ureter morphogenesis. Screening for mutations in BMP4 and FOXC1 genes in humans so far identified a mutation in three of seven patients with complex malformations including VUR (301). A translocation disrupting the ROBO2 gene was identified in a patient with high-grade reflux, and additional two families with ROBO2 missense mutations were identified in a large screened cohort (302,303). ROBO2 encodes the receptor for the SLIT2 ligand that regulates ureteric budding, thus controlling kidney morphogenesis. Recently sequenced exons and splice sites of *GDNF*, *SPRY1,* and *RET* and upstream conserved promoter elements of *RET* from a cohort of 122 unrelated living patients with multiple kidney anomalies revealed novel or rare deleterious mutations in *GDNF* or *RET* in six unrelated patients, some with defects not previously associated with *RET* in humans. A novel mutation, *RET-R831Q*, was identified in a patient and an affected relative with duplicated collecting system. Two patients with *RET-G691S* polymorphism harbored additional rare nonsynonymous variants in *GDNF* (*R93W*) and *RET* (*R982C*). The patient with double *RET-G91S*, *R982C* genotype, had multiple defects including renal dysplasia, megaureters, and cryptorchidism. In this study, 3% to 5% of living CAKUT patients had mutations in the GDNF-RET pathway (304).

VUR is also associated with heritable syndromes such as Hirschsprung disease, Apert syndrome, branchiootorenal syndrome, Townes-Brocks, and renal-coloboma syndrome

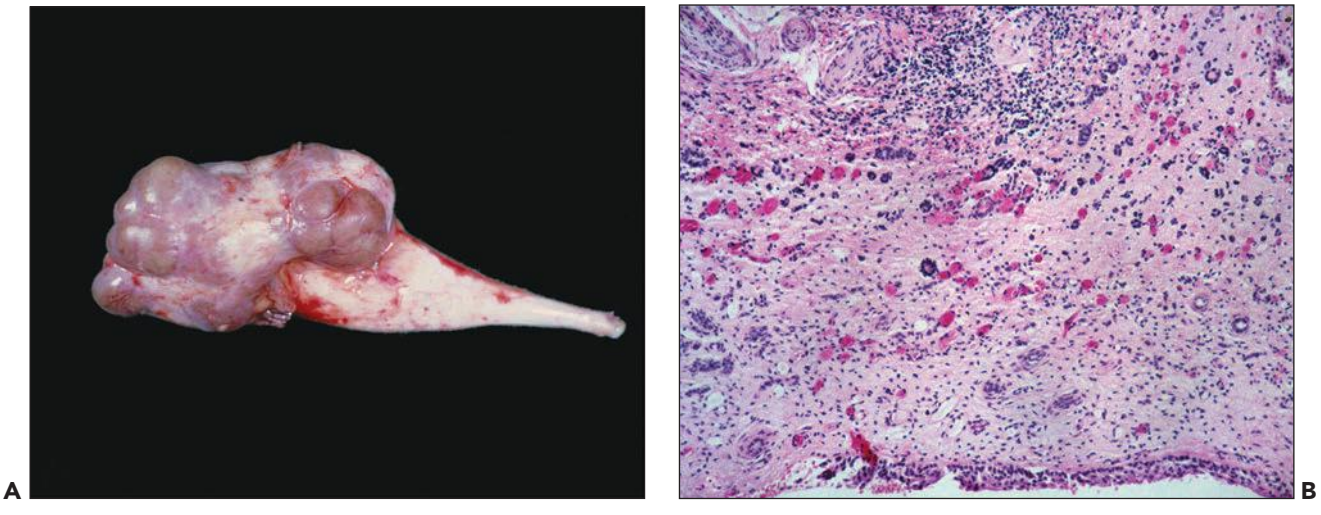


FIGURE 24.56 **A:** Atrophic kidney with cortical scars from a young boy with history of hypertension associated with nonfunctioning left kidney. **B:** Histologically, the scar consists of atrophic tubules, chronic inflammatory cells, and absent glomeruli. (H&E; ×400.)

suggesting that mutations in diverse genes may give rise to the same clinical condition (VUR and RN) and that interaction with more than one gene may be required (274,291,292,304,305). UVR is a genetically heterogeneous entity in humans, and most studies do not apply to isolated, primary reflux but are discovered in patients with either severe reflux or associated with other renal anomalies. Therefore, it is now apparent that the high frequency of VUR is likely the effect of varied mutations in many different genes that participate in the normal formation of VUJ and the distal ureter as discussed below under pathogenesis.

Reflux in the Allograft Kidney

Allograft kidneys are also known to be associated with RN. The ureter is usually simply implanted by a new ureterocystostomy. In 146 consecutive allograft nephrectomies, 4 adults were identified that had characteristic sharply demarcated cortical scars with tubular thyroidization, global glomerulosclerosis, and significant chronic inflammation (306). All four patients had recurrent infection, and two were radiologically confirmed using dimercaptosuccinic acid (DMSA) scans. Donor kidneys at the time of implantation were normal; therefore, these scars are attributed to intrarenal reflux in the allograft kidney. In these series, at least one of four patients had hilar FSGS. In pediatric patients, two children transplanted for renal dysplasia were retrospectively found to have sustained at least one urinary infection, but one (a boy) had not been on antibiotic prophylaxis for more than 3 years, while the second (a girl) was on chemoprophylaxis for over 3 years after her infections. Both had evidence of VUR in the transplanted kidney, and DMSA scan and cystogram showed de novo reflux (307).

Sterile Reflux

Sterile reflux was described by Hodson and Edwards in 1960 (307). They performed their studies in the multipapillary piglet model and based their conclusions on radiologic evidence of renal scarring induced by sterile reflux. Subsequently, Ransley and Risdon argued against a role for sterile reflux in the same model, because they found that scarring occurred only when infection was present. It was also shown that children with reflux in utero and renal scarring at birth do not develop new scars unless infections intervene (308). Others argue that absence of documented infection in humans is not proof of absence of infection, and it is highly likely that brief periods of infection will never be documented. Nonetheless, the question whether VUR without infection may cause renal damage does not appear to have been completely resolved to this day, perhaps because VUR is not always easily demonstrable in children and/or because of how clinicians worldwide may define VUR. However, new insights from genetic studies that show genetic predisposition for reflux may resolve this line of investigation in the future. Renal biopsies from children presumed to have sterile reflux are unlikely to be performed except if reflux is complicated by significant proteinuria. In the latter case, biopsy may reveal minimal inflammation and multifocal interstitial fibrosis and tubular atrophy with globally or segmentally sclerotic glomeruli. If VUR, including sterile reflux, presents with nephrotic syndrome, it is prudent to consider secondary FSGS known as hyperfiltration FSGS caused by reflux.

Reflux in Pregnancy

The existence of physiologic reflux in normal individuals, for example, pregnant women, has been investigated and thought to be due to hydrodynamic changes. During pregnancy, physiologic hormonal changes may alter ureteral peristalsis and tortuosity and cause lateral displacement of the intravesical portion of the ureter by the gravid uterus and urine reflux. Asymptomatic bacteriuria affects 7% of pregnant women, and pyelonephritis is reported in as many as 20% to 40%. Women with preexisting reflux and history of childhood UTIs are at higher risk for developing symptomatic bacteriuria and pyelonephritis compared to those without reflux, and they have an increased risk for fetal loss. In a series of 345 pregnancies, 137 women had reflux (about 1/3) (309,310). Twelve percent of these had fetal loss, and 39% had maternal complications with increased serum creatinine beyond 1.2 mg/dL. The risk was related to renal scarring, while women with VUR and no scars had insignificant complications.

Reflux and Proteinuria

Occasionally, patients with VUR will develop glomerular proteinuria. Renal biopsy may reveal FSGS, and the question is whether this is coincidental or a consequence of reflux. Review of our files shows that FSGS secondary to reflux is indeed rare, even though multiple clinical and experimental studies in the 1970s and 1980s documented the role of reflux in glomerulosclerosis (311). A Japanese study has shown moderate increase of glomerular capillary length in children with VUR and FSGS that had good prognosis and a three times increased capillary length in cases with poor prognosis suggesting that marked lengthening of glomerular capillaries in young patients with VUR is a compensatory reaction to hyperfiltration. Tuft adhesions to Bowman capsule and podocyte detachment were primarily found in patients with poor prognosis (312).

Pathogenesis

Study of VUR pathogenesis has shifted from clinical and experimentally induced VUR in animals to genetically engineered mice and cocultures of metanephric mesenchyme with ureteral bud tissue. This discussion will focus on the development of the ureter, its orifice, and the detrusor muscle at the trigone (313,316).

Development of the Ureter

The ureter is a derivative of the ureteric bud (UB), an outgrowth of the wolffian duct (WD), which in humans develops at about 5 weeks of gestation as an outgrowth from the base of the WD (Fig. 24.57). Normally, only one ureteric bud forms from each wolffian duct, thus only one ureter from each UB, thought to be restrained by lateral inhibition mediated by complex signaling from the surrounding metanephric mesenchyme (MM), a process called induction. The ureter undergoes significant remodeling at this stage, which involves separation from the WD and insertion and migration into the trigone. At least five important genes are involved in this process: PAX2, RET, GDNF, WT1, and EYA1 (316,317). Pax2 is highly expressed in the mouse mesonephric mesenchyme and thought to be responsible for the development of the mesonephric duct. Mice with homozygous Pax2 null mutation lack ureters, kidneys, and genital tract, and heterozygous mice have small kidneys. cRet is a tyrosine kinase receptor that first appears in the

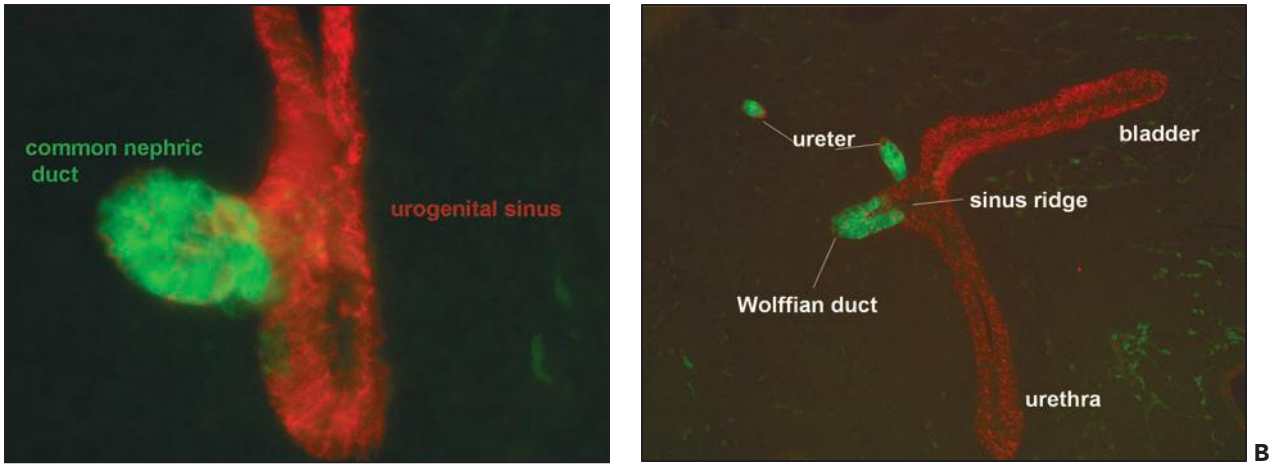


FIGURE 24.57 Development of the ureter in the Hoxb7-Gfp mouse that expresses green fluorescence protein (Gfp) in excretory duct epithelia. A: E11 mouse: The common nephric duct (stained green with Gfp) appears as a short extension of mesenchymal cells connected to the early urogenital sinus (stained red with E-cadherin). **B:** E13 mouse: A normal ureter is initially connected to the wolffian duct (urothelium, ureter, and wolffian duct are stained green with Gfp). As formation of the bladder proceeds, the ureter separates. The figure illustrates the close interaction between the wolffian duct, the ureter, and the sinus ridge, the raised portion of the dorsal urogenital sinus where insertion of the ureter takes place. (Courtesy of Cathy Mendelsohn, Columbia University School of Medicine, New York.)

pre-bud phase in wild-type mice. Later, cRet is expressed at the tip of the ureteric bud. In Pax2 mutant mice, the caudal end of the wolffian duct does not develop, and cRet is absent. As soon as the ureteric bud forms, it invades the surrounding mass of mesenchymal cells, and epithelial components start forming simultaneously with induced dichotomous branching of the ureteric buds. Ureteric bud outgrowth requires Wt1 expressed by the metanephric mesenchyme. Absence of Wt1 results in renal agenesis. Ureteric buds from cRet−/− null mice fail to branch when cocultured with mesenchyme (MM). However, branching is restored by glial-derived nerve growth factor (GDNF) and/or a transcription factor expressed in the mesenchyme called EYA1, which is thought to drive GDNF expression. As the ureteric bud continues to branch, many more molecules coordinate proper contact with the mesenchyme, and new nephrons form at the tip of the branching segments. These molecules bring both inductive and inhibitory signals and include Wnt11 that regulates GDNF, Emx-2, and TGF-β superfamily members, such as TGF-β1, BMP2, and BMP4. BMP4 is highly expressed in the mesenchyme surrounding the trunk and major branches of the ureteric bud, but it is not expressed at the periphery. Mice with null BMP4 mutations develop duplicate ureters and VUJ obstruction as well as hypodysplastic kidneys. BMP4 is thus thought to facilitate proper ureteric bud branching and inhibit ectopic or duplicate ureters. Other genes that assist in the process are those involved in program cell death, for example, BCL-2 and angiotensin 2 receptor (AT2) (315). In vivo and in vitro studies have shown that AT2 promotes apoptosis of the undifferentiated cells that surround the branching of the ureteric bud. Mice with AT2 null mutations develop VUR and duplicate ureters associated with renal malformation. It is proposed that AT2 mutation leads to inappropriate apoptosis in the mesenchyme and misguided caudal or lateral ectopias of the ureteric bud leading to VUR. These studies are influenced by hypotheses known as the "Bud Theory" proposed by Mackie and Stephens that postulated that VUR may be due to ectopic launching of the ureteric bud and loss of the right tract that would allow the developing ureter to enter correctly into the corner of the bladder base, the trigone (317). By day 33 of gestation in humans, the wolffian duct below the ureteric bud dilates and becomes the common excretory duct (urethra). The origin of the ureteric bud enters directly into the bladder by day 37, to become the ureteric orifice. Thereafter, the orifices migrate cranially and laterally to later be absorbed by the bladder at the primitive trigone. The "Bud Theory" assumes that a single embryonic mistake may explain both VUR and associated renal malformations, collectively called CAKUT (congenital anomalies of the kidney and the urinary tract). This speculation gains support by recent studies by Jain et al. that show that Ret has pleomorphic effects and intergenic interactions that may depend on which of the two major isoforms (Ret 9 and Ret 51) is activated. For example, the Ret hypomorphic mouse embryo with a mutation in Ret-PLCgamma docking tyrosine site shows complete collecting system duplication (Fig. 24.58) (318).

Development of the Trigone

The portion of the ureteric bud that lies outside the kidney will become the ureter. Distal ureters join the bladder at the trigone, defined as the triangular region of the mucosa and muscular wall of the bladder that includes the ureteral orifice. Primitive trigone forms at about 4 weeks of gestation, but ureter development will continue for weeks until between 10 and 14 weeks of gestation the ureter becomes a muscularized tube. During the end of this stage, the submucosal course into the bladder begins.

There is little known of the molecular signaling that underlines the initial process of the trigone that precedes the

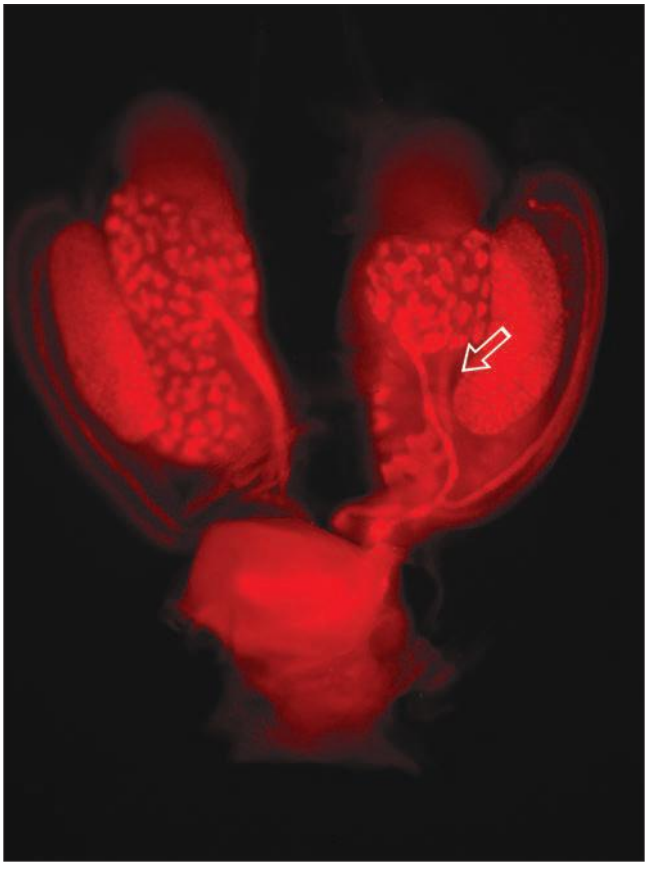


FIGURE 24.58 Whole mount E-cadherin immunofluorescence staining of urogenital tract of a midgestation Ret hypomorphic mouse embryo with a mutation in Ret-PLCgamma docking tyrosine site. There is complete duplication of kidneys and ureters bilaterally (*arrow*). Laterally to branching kidneys, the gonads are shown. These mice also have abnormalities in UVJ formation and ureter malinsertion causing megaureters and reflux postnatally. (Courtesy of Sanjay Jain, Washington University School of Medicine, St Louis, MO.)

vesicoureteral valve formation. A role for retinoic acid receptors a or b (Rara, Rarb) and vitamin A is suggested from work in Rara–/– and Rarb–/–null mice that develop incorrectly positioned distal ureters, hydronephrosis, and megaureters. Vitamin A regulates branching morphogenesis through the tyrosine kinase receptor Ret, which as mentioned above is important for UB branching. In Rara–/– and Rarb–/– mice, vitamin A and Ret mediate displacement of the UB, by controlling epithelial expansion of the base of the wolffian ducts. It is proposed that vitamin A and cRet signaling modulate the formation of the "trigonal wedge" (313). Independent studies in the HoxB7/Ret transgenic mouse have confirmed the significance of cRet in distal ureter morphogenesis (319). Thirty percent of these mice that overexpress Ret have VUR at postnatal day 1 and a shorter intravesical portion of the ureter compared to wild-type mice. Ureters are grossly dilated, and kidneys are small.

In contrast to the remainder of the bladder that derives from ectoderm (epithelial and mesenchymal cells of the urogenital sinus), the trigone is thought to derive from precursors of the mesoderm via the mesonephric duct and epithelial cells of the ureteric bud. Tissue recombination experiments show that stromal-epithelial interactions underline formation of the trigone, but it is not yet clear which precise cell types are required for proper induction (320). Independent mouse work has shown that a transient membrane (Chwalla membrane) seals the distal end of the ureter during development. Following successful insertion of the ureter into the bladder, Chwalla membrane ruptures to allow urine flow from the ureter into the bladder. There is no evidence that this membrane contributes to the valve mechanism, but temporal and spatial coordination of all elements in this location contribute to distal ureter and trigone development (321,322).

Clinical Management, Prognosis, and Therapy

The ultimate goal in the clinical management of patients with mild to moderate VUR is to prevent renal scarring. About 15% of children with prenatal hydronephrosis do not resolve postnatally; irrespective of ultrasound findings prenatally, about 1/3 will have grade I to II VUR, 1/3 will have grade III, and 1/3 will have high-grade VUR (III to IV). Therefore, it is recommended that children born with high-grade VUR, hydroureter, or bladder abnormalities be followed with VUCG postnatally. Management also includes screening of siblings (323).

In the last several years, there has been a serious effort to apply evidence-based criteria for managing patients with VUR, a task that has proven difficult to achieve (323). A particular subject of controversy is antibiotic prophylaxis to prevent infections and decrease the risk of renal scarring. Overall, it appears that a relatively small number of children with VUR have concomitant infection, ranging between 7.5% and 12%. In the majority (58%) of cases, reflux resolves with medical management (324). Persistence of reflux is directly related to the grade of reflux at the time of initial diagnosis. Severity of disease depends on laterality (unilateral vs. bilateral). Antibiotics are recommended in children with reflux that fails to resolve (325). In high-grade reflux, medical management has been less successful than surgery (326). However, the literature is unclear whether surgery prevents ESRD in severe cases (327). It is concluded that most children with ESRD have severe disease in infancy, and no surgery or medical management can prevent ultimate progression. VUR-associated ESRD accounts for about 25% of all children with ESRD, but one must not lose sight of the fact that most children diagnosed with reflux have a good prognosis and rarely need surgical intervention, or even antibiotic prophylaxis (324–327).

Previously established guidelines for evaluating and treating children with VUR were recently critically assessed (323). At least two clinical trials to address the controversy on antibiotic prophylaxis were designed: the PREVENT study in the United States and the Swedish reflux trial. In the PREVENT study, 576 children were randomized to receive trimethoprim-sulfamethoxazole or placebo. Only 6% had an absolute reduction of UTI. In the Swedish study, 203 children with grade III to IV reflux and history of UTI were randomized, and those that did not receive prophylaxis were three times more likely to develop a febrile UTI compared to children maintained on antibiotics. This effect was more pronounced in girls than in boys. These studies and review of

20 additional randomized trials of 2324 children concluded that antibiotics did not appear to significantly reduce renal scarring. In addition, the review found that many studies were not adequately designed to avoid detection or reporting bias (323). Both the PREVENT and Swedish trials failed to find statistically significant differences. Therefore, the controversy of whether to use or not use antibiotics prophylactically continues to be unresolved, but the data demonstrate that antibiotics are less effective in preventing UTI, than previously assumed.

An important aspect of VUR with detectable abnormalities at the VUJ is surgical intervention, either ureteroneocystostomy or endoscopic injection of biomaterials. Ureteroneocystostomy has high rate of success in excess of 95%. This procedure can be done with open surgery or minimally invasive approaches such as laparoscopic or robotic techniques. It is a highly effective treatment with immediate results. Endoscopic injections have variable results indicating methodological heterogeneity. Some groups report high success rate after a 4- to 6-week follow-up, and others find high recurrence rate at 2 years following successful injections (323). It seems that there is disagreement on what constitutes successful endoscopic correction. For example, in North America, successful injection is defined by absence of reflux; in the Swedish trial, success was defined as presence of grade I to II reflux.

Finally, which children should be treated surgically and when is currently clarified in published guidelines by the American Urological Association in 2010 and the National Institute for Health and Clinical Excellence of Great Britain in 2007 (277). Patient age, sex, febrile UTI, grade of reflux, and presence of renal scarring are important parameters in deciding treatment options.

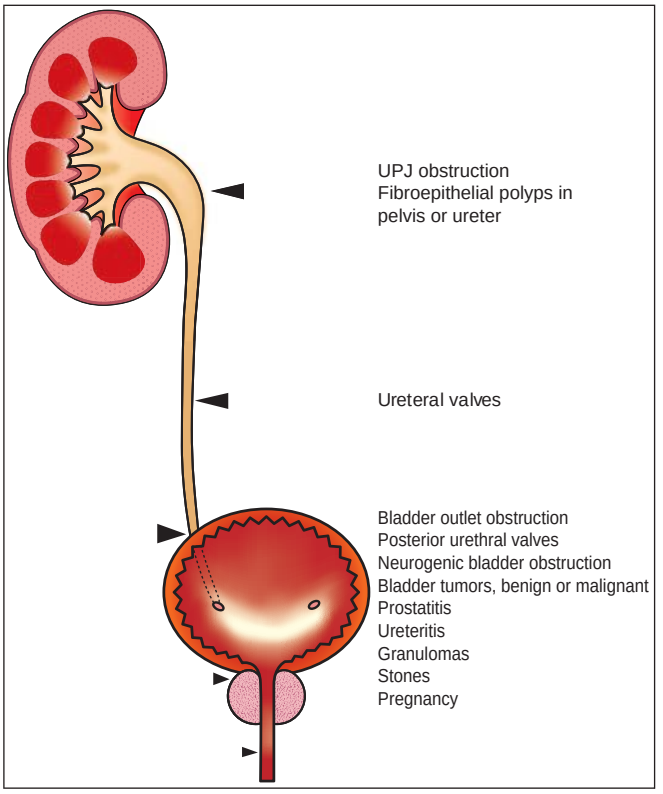


FIGURE 24.59 Causes of hydronephrosis and obstructive nephropathy.

HYDRONEPHROSIS AND OBSTRUCTIVE NEPHROPATHY

Introduction and Terminology

Hydronephrosis describes pelvic dilation due to obstruction of urine flow and back pressure of accumulated urine that distends the pelvis and/or the calyces secondary to obstruction. In contrast to RN that is defined as reversal of urine flow without obstruction and is mainly due to incompetent VUJ, urine flow obstruction congenital or acquired occurs at any level along the length of the ureter including the UPJ. UPJ obstruction is the most common cause of hydronephrosis in children and may occur as an isolated defect or coexist with stenotic posterior urethral valves, incompetent VUJ, and duplicate or crossed ureters. Ureteral diaphragms (false valves); polyps in the renal pelvis and/or renal stones; tumors derived from transitional epithelium anywhere in the genitourinary system; inflammation, such as ureteritis, urethritis, and prostatitis; pregnancy; and spinal cord damage (neurogenic bladder paralysis) may also cause hydronephrosis (Fig. 24.59). Hydronephrosis may be unilateral or bilateral, complete or incomplete, and occasionally, functional and not physical in nature. Urine flow from the kidney to the bladder is initiated at the pelvis-kidney junction with "pacemaker" cells at the base of the papilla. The coordination of a contractile wave is called pyeloureteral peristalsis and depends on the integrity of the "pacemaker," pelvic and ureter smooth muscle, and neuronal control at UPJ. Malfunction of any of these can cause obstruction. An example of functional obstruction without anatomic substrate is severe polyuria overwhelming the pyeloureteric peristalsis. If acute obstruction is relieved (e.g., stone removal), recovery is complete. If obstruction is sustained for long time periods, it leads to compression of the pelvis, calyces, and renal cortex. The damage to the kidney is referred to as obstructive nephropathy. Obstructive nephropathy is distinguished from pyelonephritis by the unique presence of infectious agents in the latter.

Ureteropelvic Junction

Incidence and Clinical Presentation

The incidence is estimated to 1 in 500 fetuses. Most children with UPJ are now diagnosed by maternal ultrasound. Excretory urography is one of the most important tests to evaluate obstructive nephropathy postnatally. This test provides an estimate of function in the involved side as well as normality or absence thereof in the contralateral kidney. Most reports find UPJ more common in boys and on the left side. The most common presenting sign is pain followed by infection and hematuria. Hematuria after minor trauma is a classic presentation, but rupture of the kidney following forceful blunt trauma is known to happen. Repair of the ruptured kidney to salvage function may be attempted. About 10% of kidneys diagnosed with UPJ have poor function at presentation, and a nephrectomy is performed. Fifteen to 30% of children demonstrate UPJ in both kidneys. Hypertension accompanies severe UPJ obstruction.

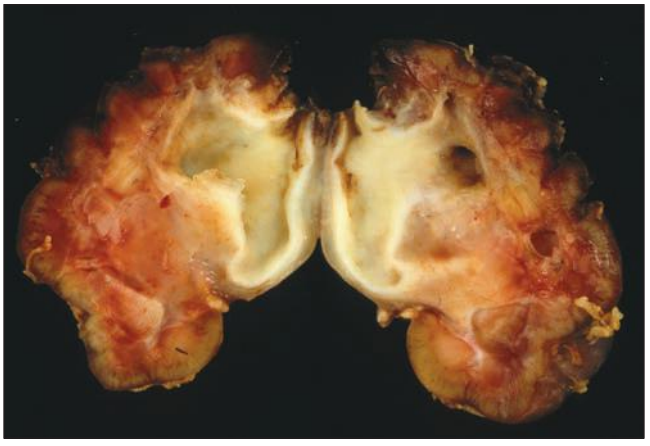


FIGURE 24.60 A 5-month-old boy with congenital right kidney UPJ obstruction. Bisected kidney shows massive pelvic dilatation.

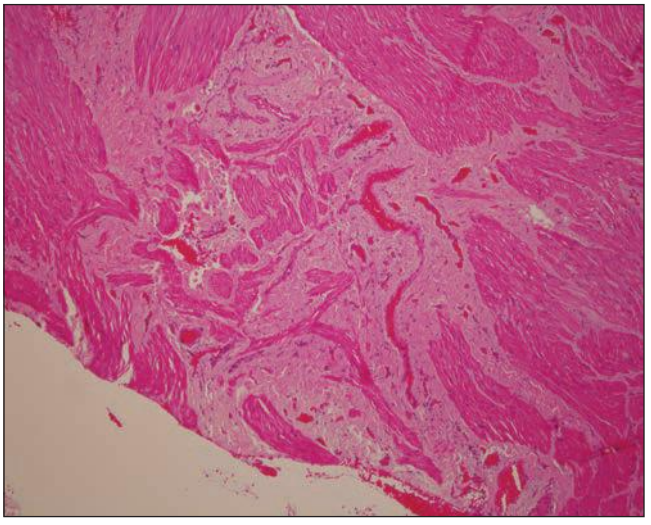


FIGURE 24.62 Pelvic muscle in UPJ obstruction. Smooth muscle fibers are randomly arranged. (H&E × 200.)

Gross Pathology and Light Microscopy

Normal UPJ is funnel shaped and, microscopically, is composed of an orderly arranged muscle layer and submucosal collagen. Kidneys with UPJ obstruction are invariably hydronephrotic (Fig. 24.60). Above the narrow UPJ, only the renal pelvis may be dilated initially, thought to provide more compliance to the accumulating pressure and preserving kidney function. In severe cases, the entire kidney becomes atrophic with cortical thinning. Histologically, early lesions show tubular dilation, followed by atrophy and interstitial fibrosis and glomerulosclerosis (Fig. 24.61). Inflammation is invariably present, but there are no grossly visible cysts. Presence of cystic lesions is a feature suggestive of renal dysplasia developing on the grounds of congenital UPJ obstruction. At the time of pyeloplasty, tissue from obstructed UPJ examined histologically shows disarrayed smooth muscle fibers and focal chronic inflammation (Fig. 24.62). Smooth muscle layers are orderly arranged normally. Renal pelvis smooth muscle layering appears to be important in the pathogenesis of UPJ and was experimentally studied by inducing partial UPJ obstruction in the rabbit. Obstruction increases muscular thickness and the collagen to muscle ratio at this site. Reversal of partial obstruction normalizes this ratio (see under pathogenesis below). In summary, pelvic and calyceal dilatation are the predominant features in UPJ. Renal dysplasia may be present histologically, particularly in kidneys with multiple cysts.

Below the narrow UPJ opening, the ureter may and often does assume a normal caliber, except when other concurrent anomalies are present, for example, obstruction of posterior urethral valves. The example in Figure 24.60 represents the most severe end of the spectrum of UPJ obstruction. However, mild and moderate obstruction is more frequent. In such cases, renal function may be adequate for long periods of time, and biopsy is rarely performed unless renal function is deteriorating. An example of partial UPJ obstruction is shown in Figure 24.63. There is apparent tubular regeneration in this

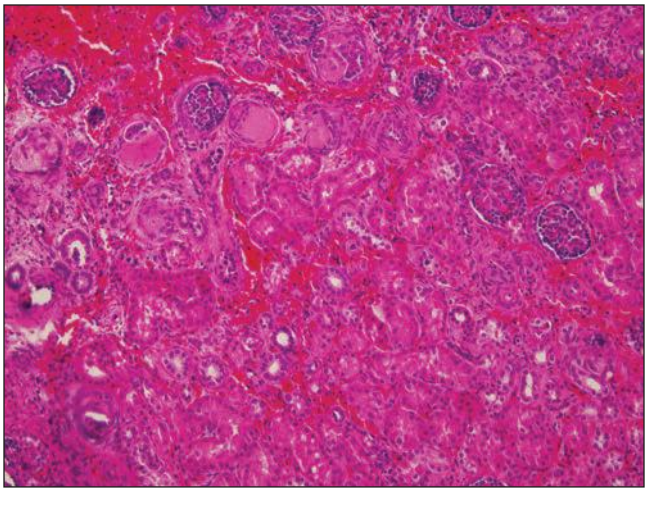


FIGURE 24.61 Sections from UPJ obstruction show renal cortex containing globally sclerosed glomeruli. (H&E × 400.)

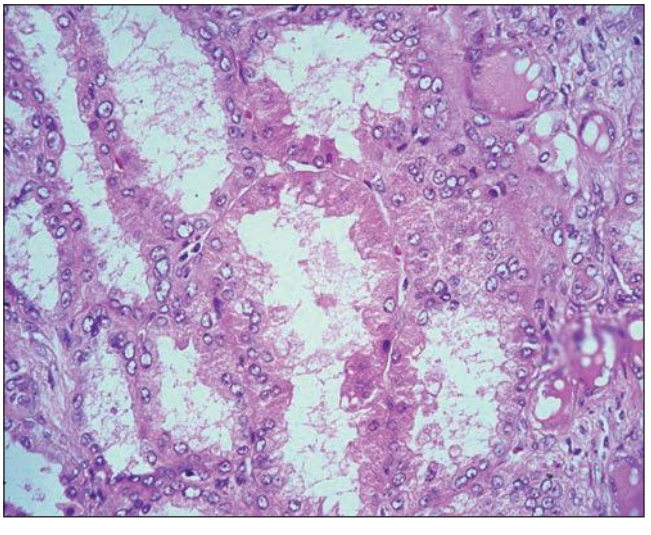


FIGURE 24.63 Tubular regeneration in partial UPJ. There is profound tubular epithelial cell proliferation and tubular dilatation. (H&E × 400.)

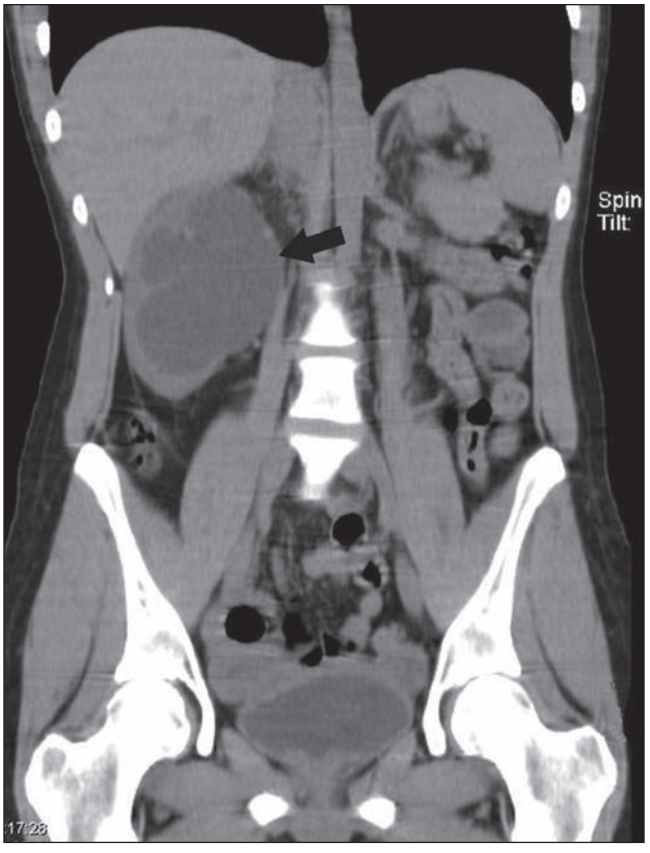


FIGURE 24.64 A 24-year-old woman with marked right kidney hydronephrosis and dilated pelvis secondary to UPJ (abdominal CT without contrast).

biopsy but no glomerulosclerosis. Objective criteria for surgical intervention in UPJ obstruction have been sought for several decades. CT is routinely utilized to evaluate severity of UPJ (Fig. 24.64). Histologic assessment at the time of pyeloplasty and grading of fibrosis scores are performed in some centers. These are as follows:

Grade 1: No histologic abnormality
Grade 2: Occasional glomerulosclerosis and minimal tubular atrophy
Grade 3: Variable, but generally limited, glomerulosclerosis and moderate interstitial fibrosis and tubular atrophy
Grade 4: Severe changes, including findings of renal dysplasia, >20% glomerulosclerosis, and extensive tubular atrophy and fibrosis

Grades 1 and 2 have excellent prognosis and correlate with good renal function assessed by radionuclide studies. Grade 3 has the poorest correlation perhaps because biopsies in this category have great histologic variability. Grade 4 biopsies project poor function (328).

Differential Diagnosis

Occasionally, obstruction in the renal pelvis will not be due to UPJ, but presence of fibroepithelial polyps (fibrovascular or densely fibrous polypoid lesions covered by transitional epithelium) (Fig. 24.65). These polyps are detected radiologically and appear as solitary tumors, cylindrical, sessile, or frond-like. Rare cases of multiple and bilateral lesions have been reported (329). Polyps are usually smaller than 5 cm and benign, but larger polyps with malignant transformation were reported. It is thought that fibroepithelial polyps are either congenital or acquired lesions that develop as a result of chronic urothelial infection, inflammation, or obstruction. Similar to UPJ, most common presenting signs and symptoms are hematuria and/or flank or abdominal pain. Urinary frequency, dysuria, and pyuria are less common findings. Fibroepithelial polyps can occur in newborns and adults older than 70 years but commonly present in the third to fourth decade, with a male to female ratio of 3:2. Approximately 62% of ureteral fibroepithelial polyps occur at the ureteropelvic junction or upper ureter, but they may also be found

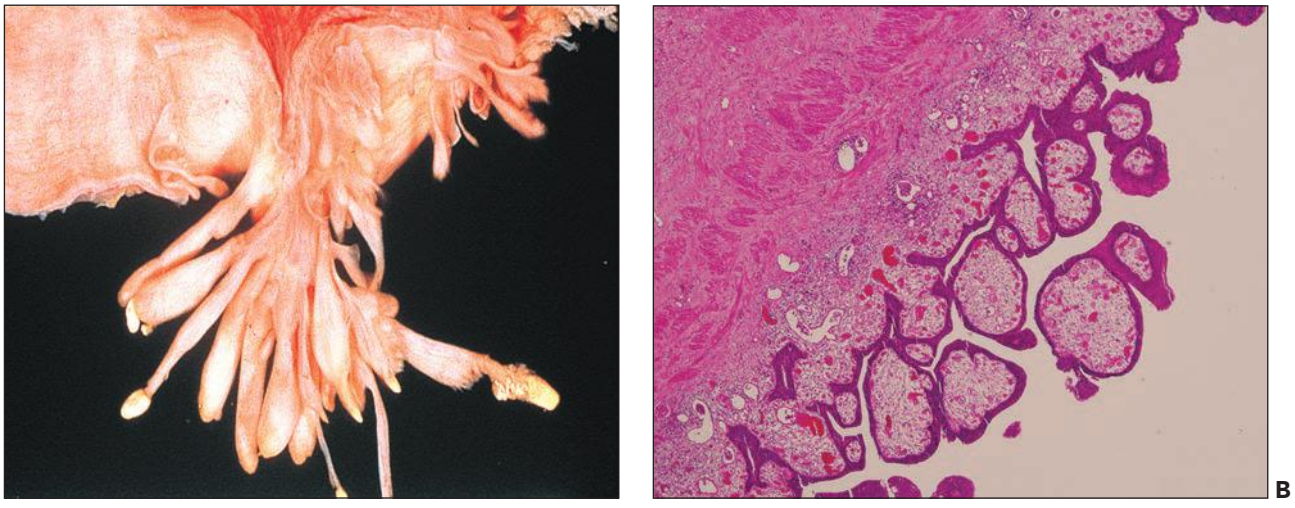


FIGURE 24.65 **Fibroepithelial polyps may cause UPJ obstruction. A:** The proximal ureter contains a polypoid intraluminal mass with multiple finger-like projections. **B:** Microscopically, polyps are lined by urothelium. (H&E × 200.)

in the lower ureter, posterior urethra, or bladder. Polyps in the renal pelvis have a female preponderance and more commonly occur on the right side, in contrast to polyps in the proximal ureter that have a predilection for men and the left side. Fibroepithelial polyps of the lower urinary tract occur most commonly in the posterior urethra and more often in children.

Other benign lesions of the upper urinary tract that may enter the differential diagnosis of UPJ obstruction in adults include endometriomas, fibromas, leiomyomas, granulomas, neurofibromas, hemangiomas, and lymphangiomas.

Hydronephrosis may also be due to ureteral valves (Fig. 24.66). These appear as translucent membranes and are thought to be embryonic remnants of incomplete recanalization of the ureter. In human 28- to 41-week embryos, during normal UPJ and proximal ureter development, ureters undergo temporary luminal obstruction, which subsequently resolves with recanalization of the ureter (330). At 35 weeks, the entire length of the ureter is patent, while the cloaca is imperforate at this time, presumably to facilitate withdrawal of mesonephric urine. Between 37 and 47 days' gestation, a membrane temporarily occludes the junction between the ureter and bladder, and the ureter becomes occluded through its entire length. Recanalization process appears to begin in the middle third of the ureter, and temporarily, it seems related to the longitudinal growth of the ureter. Muscularization is discontinuous and multicentric appearing to be induced by metanephric urine production. This complicated path to ureter maturation may cause incomplete resolution of temporary physiologic obstruction in individuals with ureteral valves.

In adults, hydronephrosis may be also caused by stones or malignancy (Table 24.5). An example of obstructive hydronephrosis due to recurrent high-grade urothelial carcinoma involving the distal ureter is shown in Figure 24.67. Renal cortex is thin replaced by massive global glomerulosclerosis

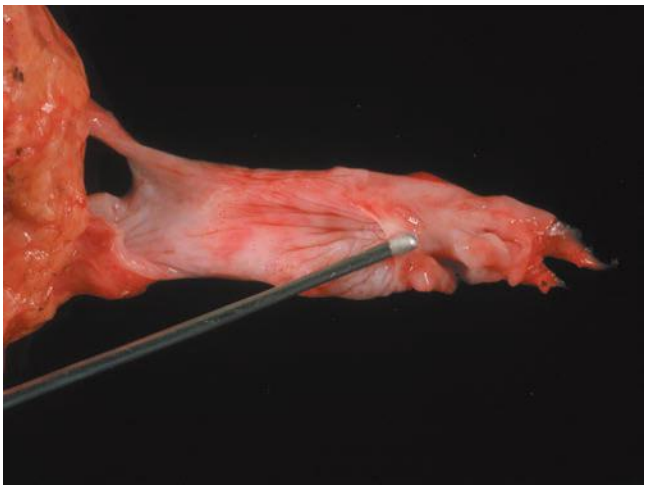
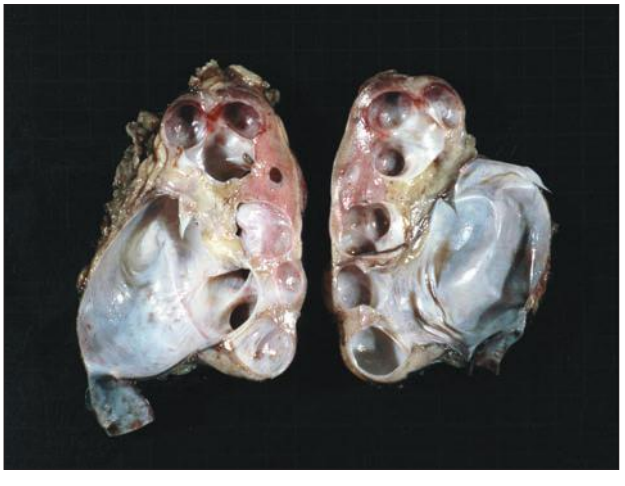


FIGURE 24.66 Ureteral valve in midportion of the ureter, from a 1-year-old girl with multicystic dysplastic right kidney.

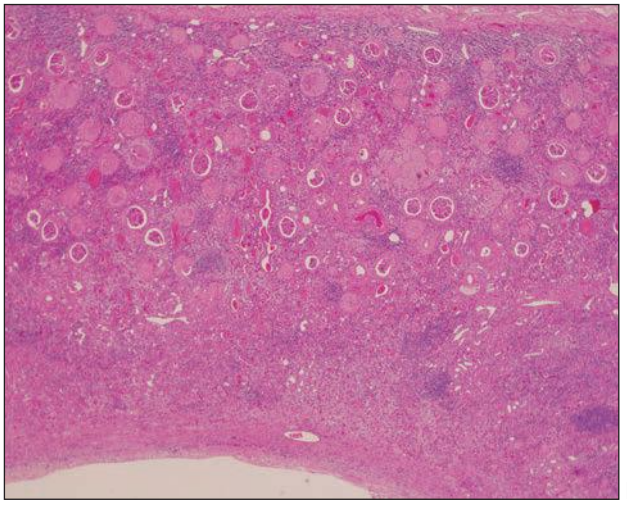
associated with chronic interstitial inflammation and tubular white cell casts.

Pathogenesis of UPJ and Obstructive Nephropathy

UPJ forms in utero at about 18 weeks of gestation. During the perinatal period, the kidney increases its urine production by about 50-fold. The renal pelvis develops rapidly then to accommodate the increased demand for urine removal by acquiring smooth muscle layers that provide adequate structural support and contractibility. Studies in mice show that the funnel-shaped renal pelvis begins right after birth. In wild-type mice, the UPJ is situated outside of the kidney proper (Fig. 24.68A). In a recombinant mouse carrying a conditional null mutation for calcineurin b isoform 1 (Cnb1), the Pax3Cre-Cnb1 recombinant mouse,



A



B

FIGURE 24.67 Obstructive nephropathy in a 65-year-old man with recurrent urinary bladder cancer involving the distal ureter. A: There is pelvic and calyceal dilatation and hydronephrosis with cortical thinning. **B:** Greater than 50% of glomeruli are globally sclerosed, tubules are atrophic, and the interstitium contains chronic inflammatory cells. (H&E × 200.)

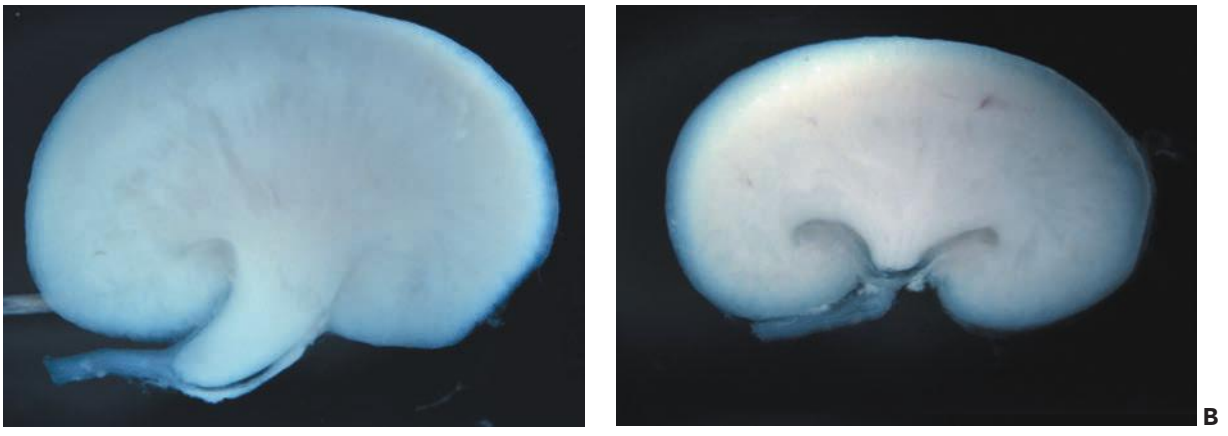


FIGURE 24.68 Control and mutant calcineurin b isoform 1 (Cnb1) mouse. A: UPJ in the wild-type mouse is funnel shaped. **B:** Mutant mouse has UPJ still tucked in the renal hilum 12 days after birth. The model demonstrates that calcineurin b1 is important in muscle development of the UPJ. (Courtesy of Feng Chen, PhD, Washington University School of Medicine, St Louis, MO.)

calcineurin function was deleted selectively in mesenchyme and smooth muscle cells of the developing kidney (331). Mutant mice have hydronephrosis and, interestingly, a flat not a funnel-shaped UPJ and lack the muscular renal pelvic extension. UPJ is still tucked in the hilum (Fig. 24.68B). The funnel-shaped pelvis fails to develop in the mutants at any age. Examination of cell proliferation in the renal pelvic wall reveals a decrease in proliferation rate in the mesenchyme where the smooth muscle cells and their progenitors reside. This study demonstrates that calcineurin is required for the proliferation of the urinary tract mesenchymal cells for the proper formation of the renal pelvis. Disruption of calcineurin function in these structures can result in malformation of the renal pelvis and ureter, leading to defective pyeloureteric peristalsis and obstructive nephropathy. At day 12, these mice are obviously hydronephrotic.

Some interesting questions that relate to maturation of the peristaltic machinery, the interactions between stromal cells, and the epithelium at the site of developing UPJ were recently redefined with unprecedented clarity. Studies show that this is a multistep process involving numerous genes early that control apoptosis of ureteral mesenchyme at the site of UB (discussed above under reflux), which becomes the ureteric epithelium and the collecting duct system. Later on, when the kidney is formed independently by differentiation of the metanephric mesenchyme, and preurine is formed, unidirectional peristalsis is thought to be initiated by specialized smooth muscle cells in the renal pelvis, known as the pacemaker cells. BMP4, sonic hedgehog (Shh), and Dlgh1 are some of the genes that participate in mesenchyme-stroma interactions, which underscore ureter maturation and effective peristalsis (332). In addition, a recently discovered gene, Teashirt-3 (Tshz3) expressed in smooth muscle cell precursors that form the wall of the developing mammalian ureter, when mutated leads to congenital hydronephrosis due to failure of functional muscularization in the top of the ureter in null Tshz3 mice (332). The hypothesis derived from these mice suggests that a signaling pathway starting with sonic hedgehog secreted by the nascent ureteric urothelium and ending with ureteric smooth muscle cell differentiation affects Tshz3 downstream of bone morphogenetic protein 4 leading to abnormal smooth muscle cell contractile protein synthesis. Furthermore, recent studies demonstrated that loss of Smad4 signaling (critical for BMP and TGF-β transcriptional responses) reduces the amount of ureteral smooth muscle and causes altered urothelial-stromal organization and UPJ (Fig. 24.69). Transcript profiling revealed altered expression of smooth muscle-specific and extracellular matrix genes prior to the onset of hydronephrosis (333). These studies suggest that if one can intervene at the stage of functional obstruction and before physical obstruction becomes apparent, renal parenchymal damage may be halted.

Obstructive nephropathy has for many decades been studied in rodents and mammals by creating physical blockage of urine through experimental unilateral ureteral ligation (UUO) complete or partial. These models allow for studying the effects of acute and chronic consequences of obstruction in the kidney parenchyma as well as the effects of obstruction release (334). Obstructive nephropathy in experimental animals induces tubular cell apoptosis, interstitial cell expansion and transformation to myocytes, infiltration by macrophages, and glomerular injury (335). Congenital obstruction is unique in that it causes accelerated apoptosis of native kidney cells destined to become nephrons, leading to kidney growth arrest (336). In adult animals, some of the mechanisms seen in newborn animals, such as apoptosis, interstitial fibrosis, and glomerulosclerosis, are shared but have different effects. An extensive number of molecules are altered in obstructive nephropathy including TGF-β, EGF, PDGF, VEGF and TNF-a, MCP-1, osteopontin, IL-1β, ICAM1, VCAM1, MMP7, HGF, HIF, and selectins (335–337). These molecules may be intermediates in the same signaling pathway (e.g., BMP7-SMAD-1, SMAD-5, SMAD-8), antagonists (e.g., MMP7 inhibits BMP7) (337), or synagonists (paracrine-autocrine loops for inflammatory cell recruitment) in different pathways, which collectively control normal nephrogenesis or tissue integrity. Disruption of such pathways has variable effects on kidney structure and function depending on the time of onset and duration of obstruction. Beyond ureteral ligation, spontaneous mutations that develop obstructive nephropathy, and more recently, genetically engineered mice have shed new light into the molecular pathogenesis of obstructive nephropathy. Furthermore, an animal model that mimics "functional"

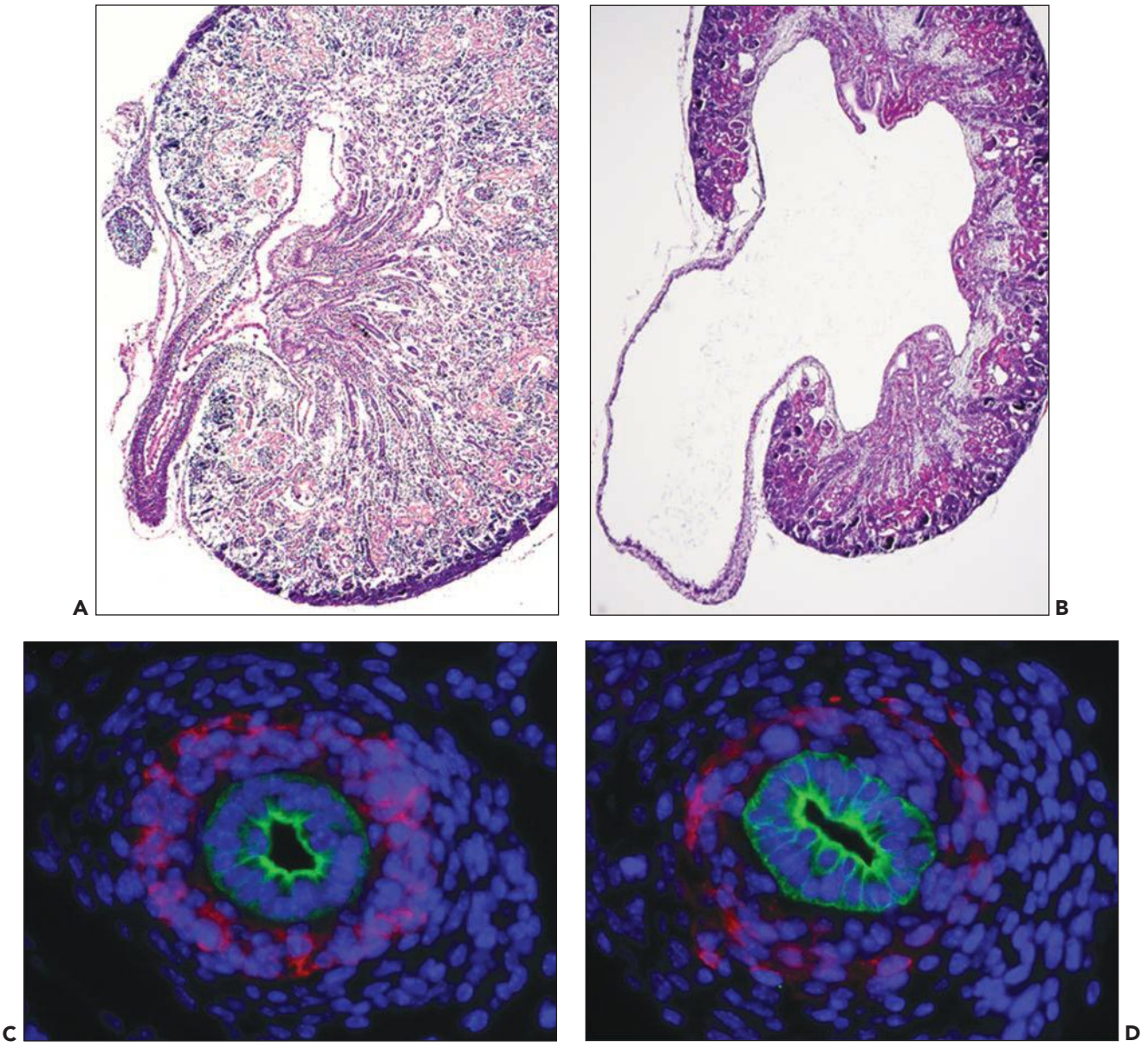


FIGURE 24.69 UPJ Obstruction and ureteral defects in the Tbx18-Cre; Smad4lox/lox mutants. A and **B:** Newborn Tbx18-Cre; Smad4lox/lox mutant mouse kidneys show UPJ obstruction and severe hydronephrosis **(B)** compared to littermate control **(A)**. **C** and **D:** Significantly reduced number of smooth muscle cells in D from Tbx18-Cre; Smad4lox/lox mutants compared to control at E15.5 **(C)**. Red signal is α-SMA (smooth muscle actin), and green signal is cytokeratin 8 labeling the urothelium. (Courtesy of Feng Chen, PhD, Washington University, St. Louis.)

obstruction in humans was developed. Twenty-seven percent of hypomorphic cystine-rich motor neuron 1 (Crim1) mutant mice show hydronephrosis in spite of normal pyeloureteric peristalsis and pelvic smooth muscle (338). In this model, Crim1 expression is decreased in pelvic smooth muscle, associated with reduced elongation of the papilla postnatally (see normal pelvic elongation in Fig. 24.68), coinciding with Crim1 expression in the immediate postnatal period. These results implicate Crim1 in normal development of the papillary smooth muscle and may explain functional obstruction in humans, which is difficult to diagnose, has subtle clinical symptoms, but may progress to CKD. Because the molecular mechanisms underscoring obstructive nephropathy in young children overlap with developmental kidney anomalies, these are discussed in Chapter 4. Obstructive nephropathy associated with infections is discussed under acute and chronic pyelonephritis in the previous section of this chapter.

Clinical Management, Prognosis, and Therapy

A significant 39% of infants with mild to moderate fetal pyelectasis have severe hydronephrosis. Ultrasound is an excellent screening tool with high sensitivity and negative predictive

value that allows avoidance of unjustified surgical treatment and unnecessary follow-up in neonates with two negative ultrasound scans. Those who will require surgery are now successfully treated in >90% to 95% of the cases (324). Minimally invasive procedures introduced in 1990 have further decreased surgical complications by approaching correction of obstructed UPJ, endoscopically or laparoscopically. However, not all cases recover renal function, and for these, there is a great need for understanding the pathogenesis of progression and to identify novel perhaps medical therapies. A major controversy still remains in selecting infants to undergo early pyeloplasty for UPJ obstruction (326). Even after successful surgery for congenital obstructive nephropathy, all patients should be periodically evaluated for hypertension, proteinuria, or renal deterioration (324).

HYPERCALCEMIA

Hypercalcemia is found in a large number of conditions many of which are associated with renal functional and morphologic lesions. With the exception of rare entities, such as familial hypocalciuric hypercalcemia (FHH), hypercalcemia almost always implies *hypercalciuria*—the excretion of excess quantities of calcium in the urine. This excretion, in turn, often leads to deposition of calcium within the renal parenchyma, called *nephrocalcinosis*, or the development of renal stones, called *nephrolithiasis*, and sometimes both. Nephrocalcinosis can be seen on occasion without hypercalcemia, as, for example, in the case of secondary hyperparathyroidism and as detailed in the section on nephrolithiasis. Nephrocalcinosis is often a more pernicious condition than nephrolithiasis since its presentation is nonspecific and may not be apparent until significant renal parenchymal damage and loss of function have occurred. Renal stones come to the fore early and usually before any significant injury because of their propensity to cause pain.

Clinical Presentation

Symptoms vary depending on the degree and duration of hypercalcemia (339–341). With mild hypercalcemia, patients may be asymptomatic. Higher levels of serum calcium can cause nausea and vomiting. With acute elevations in serum calcium, the patient may show confusion and obtundation with extracellular volume contraction and renal failure, constituting a medical emergency. There may be problems with cardiac arrhythmias, depression and psychosis, and rarely, skeletal manifestations, such as osteitis fibrosa cystica. Hypercalcemia may be marked by various acid-base disorders, notably both proximal and distal renal tubular acidoses (RTA). There is also salt wasting, potassium and magnesium wasting, as well as aminoaciduria (341,342). Defects in the ability to concentrate urine are perhaps the earliest manifestations and, indeed, may be the only recognizable abnormality in asymptomatic patients whose hypercalcemia is incidentally recognized during examination for another problem. In acute hypercalcemia associated with multiple myeloma, the severity of renal insufficiency tends to parallel the degree of hypercalcemia and severity of the resulting dehydration (343). Chronic calcium nephropathy can present as interstitial nephritis, with polyuria, little proteinuria, and a bland urinary sediment.

TABLE 24.6 Causes of hypercalcemia

- Primary hyperparathyroidism
- Malignancy-related hypercalcemia
  - Humoral
  - Skeletal metastases
- Genetic causes
  - Mutations of the calcium-sensing receptor
    - Familial hypocalciuric hypercalcemia
    - Neonatal severe hyperparathyroidism
  - Williams syndrome
  - Bartter syndrome
- Infantile or neonatal conditions
  - Iatrogenic
  - Secondary hyperparathyroidism
  - Subcutaneous fat necrosis of the newborn
- Vitamin A or D excess
- Sarcoidosis
- Milk-alkali syndrome
- Thyrotoxicosis
- Immobilization
- Medications

Causes of Hypercalcemia

Hyperparathyroidism is the cause in approximately 55% of cases of hypercalcemia (Table 24.6). Hypercalcemia associated with malignancy (humoral or osteolytic), accounting for approximately 35%, is the most common cause in hospitalized patients. After malignancy, the incidence of conditions associated with hypercalcemia declines rapidly. Other causes such as vitamin toxicity, milk-alkali syndrome, granulomatous diseases, and drugs account for <10% of cases. In some cases, hypercalcemia is clearly due to deossification of the skeleton, particularly in hyperparathyroidism and malignancy. Some conditions can be categorized as representing increased intestinal absorption of calcium. In other instances, however, the source of hypercalcemia is not clear (341–344).

Primary Hyperparathyroidism

Primary hyperparathyroidism is characterized by spontaneous, unregulated oversecretion of parathyroid hormone (PTH) leading to increased bone resorption and intestinal calcium absorption. Benign adenomas (single-gland or multiple-gland involvement) are the underlying cause in 85%, hyperplasia in 15%, and carcinoma in <1% of cases. The prevalence of primary hyperparathyroidism is approximately 1 to 4 per 1000 with a female to male ratio of 3:1. In postmenopausal women, 3% have hyperparathyroidism (341,345). The cause is usually sporadic, though primary hyperparathyroidism can also be found in some inherited syndromes, such as multiple endocrine neoplasia (MEN) 1 and 2, hyperparathyroidism-jaw tumor syndrome, and familial isolated hyperparathyroidism (345).

Patients with primary hyperparathyroidism are typically asymptomatic, with modestly elevated serum calcium detected on routine screening. Serum calcium concentrations at the time of diagnosis are usually <1 mg/dL above normal. Nearly 75% of patients have no signs or symptoms attributable to the parathyroid abnormality. Bone, kidney, and other related changes are becoming rare. When primary hyperparathyroidism was

initially described in the 1920s, patients typically were diagnosed with severe bone disease, *osteitis fibrosa cystica*, and pronounced hypercalcemia. These patients were rare then and are very rare today, particularly in developed countries (346).

Physiology of Parathyroid Hormone

PTH secretion by the parathyroid glands is regulated in a negative feedback fashion by serum concentrations of ionized calcium (347,348). Regulation of PTH is through the calcium-sensing receptor located on the surface of parathyroid cells (349). PTH is produced as a preprohormone and is then proteolytically converted to the active 84 amino acid molecule (348,350,351). The intact hormone has a molecular weight (MW) of 9500, but it is rapidly metabolized in the liver, kidneys, and bone into two fragments. The smaller fragment (MW 3500) comes from the amino terminal portion of the hormone and contains the biologic activity, whereas the larger, MW 5500 carboxy terminal end has no activity. Both the intact hormone and the amino terminal fragment are rapidly removed from the circulation, whereas the carboxy terminal fragment has a longer half-life (90 minutes).

Renal Function

Important functional renal manifestations of hyperparathyroidism include decreased glomerular filtration rate and abnormalities in tubular function leading to decreased concentrating ability (352). Hypercalciuria is seen in approximately 40% of patients. Stones remain the most common renal manifestation of primary hyperparathyroidism, seen in roughly 15% to 20% of patients (346,353) and in approximately 7% of asymptomatic patients with hyperparathyroidism, which is a significantly higher prevalence than among the background population (1.6%) (354).

The action of PTH to increase intestinal absorption of calcium through vitamin D and bone resorption are key factors in development of hypercalcemia (355). Since PTH has a direct effect to increase reabsorption of filtered calcium in the renal tubule, enhanced tubular reabsorption also contributes to the hypercalcemia.

Diagnosis

The diagnosis of primary hyperparathyroidism is made by noncompetitive immunoassay of serum PTH levels with antibody against the full-length active molecule (peptides 1 to 84). In the presence of hypercalcemia, elevated intact PTH confirms the diagnosis of primary hyperparathyroidism, whereas in hypercalcemia of malignancy, PTH levels are suppressed or within the normal range. In idiopathic hypercalciuria, serum calcium levels are normal and PTH levels are normal, but urinary excretion of calcium is very high. In FHH, which can be confused with primary hyperparathyroidism, PTH can be elevated or normal, but calcium excretion is very low; FHH patients also typically present at a younger age and usually with a family history. Neonatal severe hyperparathyroidism is associated with extremely high serum calcium and PTH levels, at odds with the findings in other conditions associated with elevated PTH (356,357).

Treatment and Outcome

In symptomatic patients, surgical removal of the parathyroid glands is recommended and highly successful. In many asymptomatic patients, however, medical management is recommended. The National Institutes of Health (NIH) consensus conference in 2002 and more recently in 2009 (358) developed guidelines for surgery in asymptomatic hyperparathyroidism. Surgery should be considered only in patients with one of the following: serum calcium >1 mg/dL above normal, calciuria >400 mg/d, markedly reduced bone density (T score less than −2.5), creatinine clearance reduced below 30% of normal (age and sex matched), or the patient is younger than 50 years of age. In one study of the natural history of primary hyperparathyroidism, approximately 37% of patients followed conservatively over 15 years eventually met criteria for surgical intervention (359). All patients with a diagnosis of hyperthyroidism should initially be evaluated for renal calcifications by unenhanced helical CT. If calcifications are present, parathyroidectomy is recommended. If symptoms develop after parathyroidectomy, patients should be evaluated and treated similar to other patients with renal stones (358).

Pathology

A single parathyroid adenoma is responsible for hyperparathyroidism in 85% to 90% of patients (360). Adenomas occur in a single gland, vary in size from less than a gram to more than 100 g, and are made up most frequently of chief cells. Oncocytic/oxyphil cells, transitional cells, water-clear cells, or a mixture of cells make up the remainder of adenomas. A rim of normal parathyroid tissue admixed with adipose tissue cells can be seen compressed around the edge of the adenoma nodule. No adipose tissue is found within the adenoma. In very rare cases, multiple adenomas may be found. In parathyroid hyperplasia, there also is little or no adipose tissue, and any or all cell types normally found in parathyroid are present. Four-gland hyperplasia constitutes most of the cases. Most show chief or oncocytic cell hyperplasia, diffuse or nodular (360), with nodular hyperplasia predominating in older patients. Parathyroid carcinomas make up a minority, <1%, of parathyroid lesions. Similar to adenomas, they may produce a mass, but unlike adenomas, it is generally ill defined rather than a distinct nodule and shows infiltrative growth and capsular invasion. They are best recognized not simply from nuclear pleomorphism, which may be seen in benign lesions, but from the infiltrative growth pattern and evidence of metastasis.

Little information is available in the recent literature on histology of the kidney in hyperparathyroidism. The principal lesions described in older reports were those owing to nephrocalcinosis, such as might be seen with hypercalcemia of any cause, not simply that related to hyperparathyroidism (352). The most important renal lesion in hyperparathyroidism is development of renal stones and their consequences, such as infection and obstruction. Secondary pathology related to the obstructive nature of stones is described in the section on hydronephrosis.

Hypercalcemia of Malignancy

Hypercalcemia in association with malignancy has long been recognized and is primarily related to bone resorption. There are four primary mechanisms for hypercalcemia in association with malignancies.

- Humoral hypercalcemia of malignancy (HHM) is responsible for approximately 80% of cases and is due to tumoral secretion of parathyroid hormone–related peptide (PTHrP).

- Approximately 20% of cases of cancer-related hypercalcemia are related to local osteolysis by the tumor, most commonly breast cancer and multiple myeloma (361). Among the factors implicated in osteolytic hypercalcemia of malignancy are IL-1, IL-3, and IL-6, osteoprotegerin, and receptor activator of nuclear factor–kappa B (RANK).
- Ectopic 1-alpha-hydroxylation of 25-hydroxyvitamin D resulting in increased levels of 1,25-dihydroxyvitamin D occurs in <1% of cases.
- Tumors can secrete PTHrP (fewer than 1% of cases).

Many nonmetastatic tumors release parathyroid hormone receptor–like protein (PTHrP), producing the condition known as HHM. PTHrP behaves very much like PTH; it interacts with PTH receptors, leads to bone resorption, and causes a rise in urinary cyclic adenosine monophosphate (cAMP) that triggers increased tubular reabsorption of calcium (361,362). PTHrP shares structural homology with the amino terminus of PTH (residues 1 to 13), but thereafter, it diverges while retaining the ability to regulate calcium handling by binding to the same receptor as PTH. In addition to being found in tumors, PTHrP is also found in normal keratinocytes, lactating breast tissue, the brain, the lungs, the parathyroid glands, and other sites. Tumors reported to have an association with elevated PTHrP are breast cancer, squamous cell carcinoma, renal cell carcinoma, bladder carcinoma, prostate cancer, pheochromocytoma, pancreatic cancer, lung cancer, and some lymphoid malignancies (361–363). A less common cause of hypercalcemia of malignancy is tumor secretion of 1,25-dihydroxyvitamin D (364).

There appears to be no consistent site of deposition of calcium, and any segment of the nephron may be affected. In some instances, the disparity between the morphologic damage and the degree of renal failure may be impressive, with only very scanty calcification. Bisphosphonates are used to treat hypercalcemia of malignancy, but some have been shown to cause glomerular disease (365) or ATN (366).

Neonatal or Infantile Hypercalcemia

Neonatal severe hyperparathyroidism (primary) is very rare and is caused in most cases by an inactivating mutation of the calcium-sensing receptor (367). This entity is discussed in the section on FHH. Secondary hyperparathyroidism may occur in an infant whose mother's calcium metabolism goes awry during gestation. Complications occur in up to 80% of affected fetuses and include growth retardation and preterm delivery (368). During pregnancy, surgical removal of a maternal parathyroid adenoma may be indicated to avoid fetal demise. Infants usually require only supportive treatment in the neonatal period until the hypercalcemia resolves (369).

In infants, chronic renal insufficiency may result either from nephrocalcinosis leading to tubular dysfunction or from nephrolithiasis. The most common cause of infantile hypercalcemia may be iatrogenic, owing to intravenous administration of calcium usually from parenteral nutrition, and is alleviated by cessation of the infusion. Use of loop diuretics can cause altered calcium handling by the nephron in infants; cessation of diuretics will lead to resolution. In premature or very low birth weight neonates, the kidneys are not fully developed functionally, and several transport mechanisms can favor hypercalciuria, such as low citrate excretion, decreased bicarbonate reabsorption, and alkaline urine pH (368,370).

Williams Syndrome

Previously known as severe idiopathic infantile hypercalcemia (IIH), Williams syndrome (also known as Williams-Beuren syndrome) is a multisystem genetic disorder caused by a contiguous deletion in the long arm of chromosome 7, affecting a large number of genes known to include elastin and LIM 1 kinase, among several others (371,372). The approximate incidence is 1 per 10,000 births worldwide, and it is mainly a sporadic defect with a few reported cases of apparent autosomal dominant transmission (373). Patients are born small for gestational age with a characteristic facies, develop hypercalcemia and nephrocalcinosis and cardiovascular abnormalities—most often supravalvular aortic stenosis, have neurodevelopmental defects that affect hearing and speech, and have very distinctive personalities. Approximately 10% to 15% of these patients have hypercalcemia that often resolves in the first year (373,374), although the hypercalcemia may continue or recur in adulthood. Up to 50% of patients have hypercalcemia, with associated hypercalciuria.

Subcutaneous Fat Necrosis of the Newborn

In this rare form of panniculitis, there are plaques of fat necrosis, often in the buttocks and posterior trunk, in the wake of which hypercalcemia often develops. This condition occurs most often in neonates who experienced a complicated delivery (375) and usually presents in the first week of life. Characteristic nodules are seen on the buttocks, trunk, arms, and cheeks. Histologically, the lesions consist of fat necrosis with a granulomatous inflammation including histiocytes, giant cells, and needle-shaped clefts in fat cells (376). The condition usually has a benign course with resolution of the skin lesions, though hypercalcemia may develop up to several months later and can be a cause of significant morbidity and mortality (377). Transient renal insufficiency and nephrocalcinosis can develop. The underlying mechanism of the hypercalcemia is not clear, but may involve release of $1,25(OH_2)D_3$ from the granulomatous areas of inflammation, leading to increased intestinal uptake of calcium (377).

Disorders of the Calcium-Sensing Receptor

The calcium-sensing receptor (CaR) is located on a number of cell types throughout the body including the parathyroid gland, the thick ascending limb of the loop of Henle and distal convoluted tubule segments in the kidney, the mucosa of the gastrointestinal tract, osteoclasts, and some cells in the brain (349,378). The CaR located on parathyroid cells regulates the release of PTH. High calcium activates the CaR, which inhibits PTH release, leading to a reduction in serum calcium. In the kidney, CaR molecules in the thick ascending limb sense elevated calcium levels in the peritubular capillaries and reduce calcium reabsorption. Changes in water permeability of the collecting duct may also be mediated through the CaR, allowing for polyuria and less concentrated urine to help prevent calcium precipitation and the formation of urinary stones (379). Several disease conditions have been associated with mutations of the CaR gene.

Familial Hypocalciuric Hypercalcemia

Heterozygous loss-of-function mutations result in FHH (378–381). This disorder has an autosomal dominant inheritance. FHH is usually asymptomatic, characterized by hypercalcemia,

hypocalciuria, mild to moderate hypermagnesemia, mild hypophosphatemia, and normal to slightly increased PTH values (not suppressed as would be expected with hypercalcemia). The mutation in the CaR results in a decreased sensitivity of the receptor to calcium levels in the serum so that a higher calcium concentration is required for its activation, leading in most cases to mild hypercalcemia. A lack of the normal inhibition of PTH secretion by the CaR also contributes to hypercalcemia. The mutation is also responsible for the paradoxical hypocalciuria, owing to a lack of thick ascending limb CaR response to the hypercalcemia. Calcimimetics may have some utility in stimulating the receptor pharmacologically.

Hypercalcemia symptoms similar to those seen with loss of function can occur also in *individuals with inactivating antibodies* (378).

Severe Neonatal Hyperparathyroidism

Homozygous loss-of-function mutations in the CaR result in this life-threatening form of hypercalcemia (380) characterized by failure to thrive and fractures owing to undermineralization of the skeleton. The total lack of CaR in the parathyroid gland leads to markedly elevated PTH. Parathyroidectomy may be necessary for survival.

Gain-of-Function CaR Mutations

Individuals with inactivating mutations (heterozygous or homozygous) of the CaR do not typically develop nephrocalcinosis or nephrolithiasis, despite the hypercalcemia. Alternatively, gain-of-function CaR mutations lead to hypocalcemia and hypercalciuria owing to hypoparathyroidism (349,378). These mutations cause autosomal dominant as well as sporadic cases of hypoparathyroidism (381). In individuals with gain-of-function mutations, the hypercalciuria leads to an increased tendency toward developing nephrocalcinosis and nephrolithiasis (378,382). Other symptoms vary by patient; some have asymptomatic hypocalcemia, whereas others may have spasms and seizures due to hypocalcemia (378).

Vitamin A or D Excess

Prolonged ingestion of large amounts of vitamin A (in excess of 50,000 IU/d) or administration of retinoic acid as treatment for various malignancies has been shown to result in bone resorption and hypercalcemia (383–385). Vitamin A taken as a dietary supplement can build up in tissues and produce acute as well as chronic toxicity.

Hypercalcemia caused by vitamin D excess has been documented to occur in several ways. Cases of ingestion of excessively fortified milk have been reported (386,387), with some individuals developing hypercalcemia. Some hematologic malignancies are associated with elevated levels of circulating $1,25(OH_2)D_3$ 360. Whether the lymphomatous cells themselves produce the excess vitamin D or whether, as suggested by Hewison et al. (388), the vitamin D is produced by tumor-adjacent macrophages remains to be determined.

Pathologic descriptions of vitamin D excess in the older literature depict kidneys that are normal in size or enlarged, pale, swollen, and yellow. Calcification takes place in all segments of the tubules, and foreign body giant cells are sometimes seen. Tubular loss, lymphocytic infiltration of the interstitium, periglomerular fibrosis, and sclerosis may all be present (389). Metastatic calcification may be evident in the heart, arteries, lung, and stomach. Calcification may also occur around joints, sometimes in the form of calcium-containing cysts.

Granulomatous Diseases

Sarcoidosis and other granulomatous diseases, such as tuberculosis, Crohn disease, and leprosy, can be a cause of hypercalcemia and hypercalciuria owing to excess vitamin D from extrarenal conversion of $1,25(OH_2)D_3$ (390). In one study, nephrocalcinosis was found in 22% of patients with chronic sarcoidosis (391).

Milk-Alkali Syndrome

This syndrome was formerly a complication of peptic ulcer disease in patients who took large quantities of milk and absorbable alkali as therapy, which led to hypercalcemia and alkalosis. Nephrocalcinosis ensued, leading to renal insufficiency in some patients. With the advent of histamine blockers, the classic milk-alkali syndrome became rare; however, cases of milk-alkali syndrome associated with the use of calcium carbonate to treat osteoporosis or chronic renal failure became more common. In a 2005 study, 8.8% of patients with hypercalcemia were found to have the milk-alkali syndrome, the third most common cause of hypercalcemia behind malignancy and hyperparathyroidism (392). Renal pathologic descriptions are scanty, but they document foci of calcification in the tubules and interstitium with interstitial fibrosis and inflammation (393).

Defects of Tubular Handling

Although not always associated with hypercalcemia, several disorders of tubular handling are associated with nephrocalcinosis and/or nephrolithiasis owing to hypercalciuria. Abnormalities have been identified in paracellin, a tight junction protein, in phosphate transporter regulatory proteins, and in potassium channels and chloride channels. These disorders have been meticulously reviewed elsewhere (394–398). Familial hypomagnesemic hypercalciuria, autosomal dominant hypoparathyroidism, antenatal Bartter syndrome (or hyperprostaglandin E syndrome), and classic Bartter syndrome all have in common hypercalciuria, which predisposes to nephrocalcinosis and nephrolithiasis.

Pathology of Renal Calcification (Nephrocalcinosis)

Renal calcification refers to calcium deposits in the form of calcium phosphate and calcium oxalate in the kidney parenchyma. In most cases, calcium phosphate deposits are focal and may be seen in many conditions including in patients with significant proteinuria, following ischemic tubular injury, aging kidneys, and preterm babies partly because of intrinsic kidney calcium losses and partly from enhanced calcium excretion following diuretic therapy (399). Diffuse calcium phosphate crystals are particularly associated with oral sodium phosphate bowel preparations (phosphate nephropathy) and gastric bypass surgery (dumping syndrome). Calcium phosphate deposits appear purple on H + E (basophilic) and stain black with von Kossa stain and red with alizarin red, which stains calcium (red). Calcium phosphate crystals do not show birefringence under polarized light in contrast to calcium oxalate.

The earliest deposits are intracytoplasmic, but they erode into the lumen at an early stage or less frequently expand, pushing the tubular cytoplasm before them effectively occluding

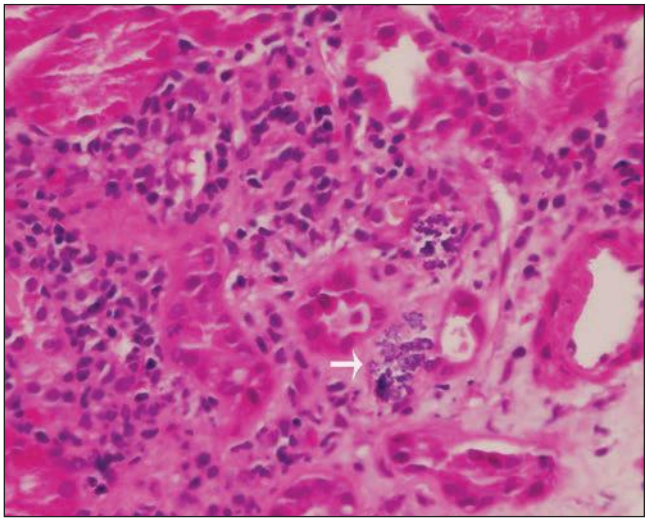


FIGURE 24.70 Granular calcium phosphate deposits (*arrow*) in nephrocalcinosis. (H&E; ×400.)

the tubule. In the early stages, the structures are pale to clear with rounded or somewhat irregular contours. As they grow, they progressively calcify and become granular and basophilic, sometimes with a faintly lamellar appearance resembling miniature psammoma bodies. The tubular cytoplasm, if it persists, becomes flattened and pale, but often, it is completely eroded away. These isolated concretions are usually confined to the tubule without surrounding inflammation. Occasionally, they may rupture through the tubular basement membrane (TBM) into the interstitium with resulting inflammatory response. Calcium deposits are then deposited in the interstitium, usually just outside tubules (Fig. 24.70). Phosphate crystals in hyperthyroidism are deposited along the basement membrane of the proximal tubules. This pattern can also be seen in neonates with hypercalciuria (Fig. 24.71).

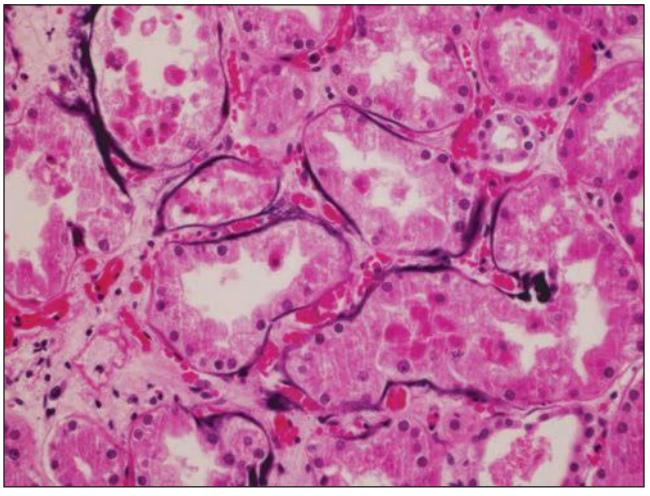


FIGURE 24.71 Basophilic deposits along the tubular basement membranes, from a neonate with acute renal failure following prolonged hospitalization. (H&E; ×400.)

Wiech et al. (399) found phosphate-containing crystals in hyperphosphatemic patients to have a shell-like appearance in contrast to hypercalciuric patients who had predominantly globular or granular (calcium type) deposits arguing that morphology can be used to distinguish the two. Calcium-containing deposits were called dystrophic in the older pathology literature and the term nephrocalcinosis referred to deposits associated with tissue necrosis or malignancy. Recent autopsy studies found kidney calcium deposits in 223 out of 12,960 autopsies; an incidence of 1.7%; 111 (49.5%) of the cases had advanced malignant neoplasms (399).

Calcium-containing deposits can be classified according to location as cortical or medullary. The patient in Figure 24.72 was diagnosed with nephrocalcinosis radiologically. Medullary sponge kidney (a distinctly radiologic diagnosis recognized by calcifications in the collecting ducts) is an example of exclusive medullary calcium deposits (see Chapter 4). Some authors use the term *crystalline nephropathies* for calcium-containing kidney deposits and include four categories: (a) calcium-containing deposits, (b) drug-induced crystals, (c) metabolic- or genetic-associated deposits, and (d) dysproteinemia (400).

Calcium Phosphate Crystals and Bowel-Cleansing Agents (Phosphate Nephropathy)

Diffuse calcium phosphate crystals are particularly associated with oral sodium phosphate bowel preparations, also called phosphate nephropathy. Renal failure with biopsy-proven ATN and nephrocalcinosis follows oral sodium phosphate solutions prior to colonoscopy (400,401). Deposition of nonpolarizable calcium phosphate crystals in this case are found without hypercalcemia or other known risk factor for the development of nephrocalcinosis. Most of these patients have normal renal function prior to the procedure and develop acute renal failure following colonoscopy. Many of the affected patients are hypertensive before the incident; other patients have conditions associated with reduction in perfusion pressure,

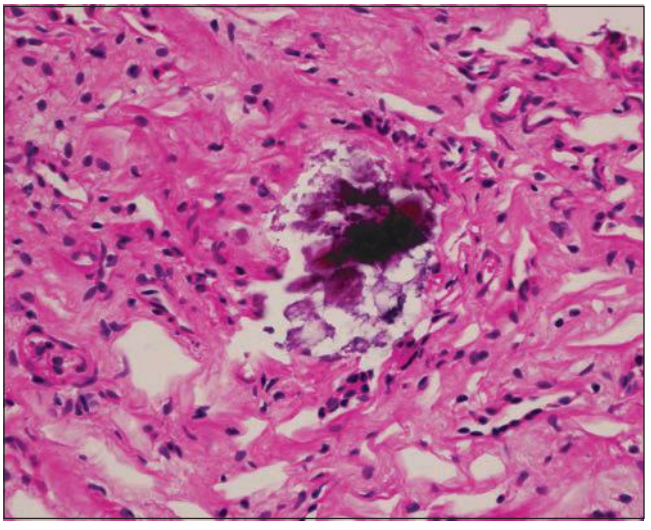


FIGURE 24.72 Crystal-like calcium phosphate deposits in the medulla. Renal biopsy from a patient with nephrocalcinosis diagnosed on ultrasound. (H&E; ×400.)

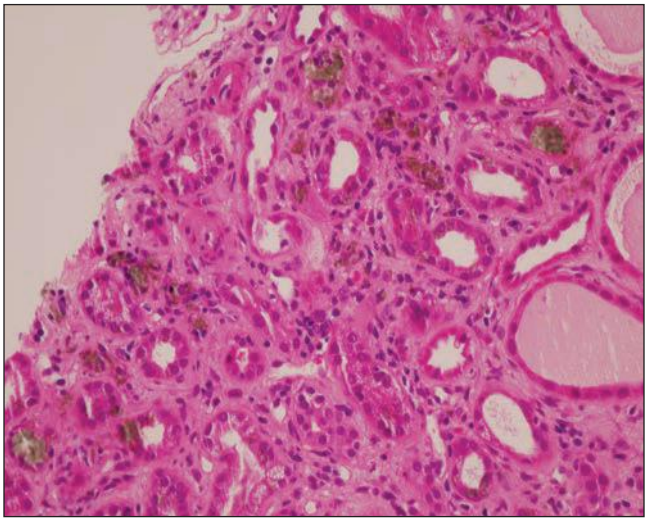


FIGURE 24.73 Phosphate nephropathy in a transplant patient who developed acute renal failure following colonoscopy. (H&E; ×200.)

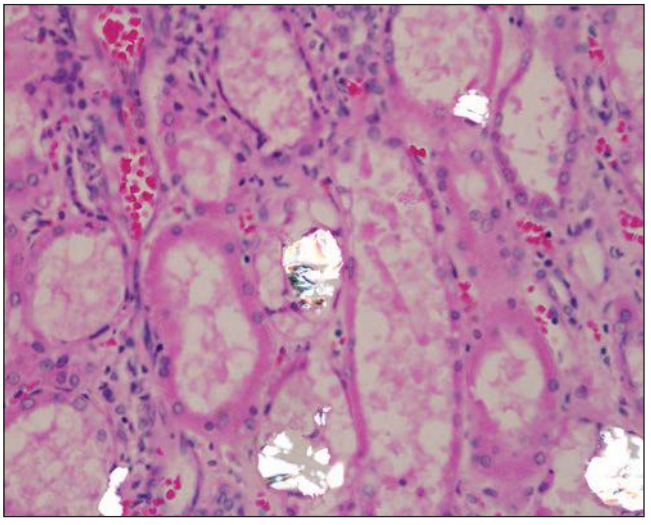


FIGURE 24.74 Calcium oxalate nephropathy. CaOx crystals appear white or colored under polarized light. Biopsy from patient with sepsis-associated acute kidney injury. (H&E; ×200.)

such as dehydration or use of diuretics. Unfortunately, in most cases, the decline in renal function is irreversible.

Calcium phosphate crystals in phosphate nephropathy have a brown color and stain black with von Kossa stain. On renal biopsy, deposits abound but with minimal or no interstitial inflammation (Fig. 24.73).

Calcium Oxalate (CaOx) Nephropathy

These deposits are often found in end-stage native kidneys and allograft kidneys (36,402). Their etiology is diverse and includes ATN or acute cell-mediated rejection, increased excretion of stored CaOx prior to transplantation, antihypertensive medications (naftidrofuryl oxalate), and recurrent hereditary oxalosis (402). Several reports have described nephrocalcinosis in AIDS patients. In many instances, nephrocalcinosis is but one manifestation of multiorgan calcification in the liver, spleen, adrenals, and lymph nodes during the course of disseminated infection with *Pneumocystis carinii* organisms (403). Nephrocalcinosis in HIV infection may be due to hypercalciuria (404), RTA, or indinavir therapy (405).

Histologically, calcium oxalate crystals are colorless on H + E or yellowish brown; they appear fan shaped, radially arranged or spiculated, and are birefringent on polarization as opposed to calcium phosphate crystals (Fig. 24.74). Acute and chronic tubular injury and inflammation occur in early stages of CaOx deposition, sometimes including a giant cell reaction; later, there is tubular atrophy and interstitial fibrosis. The role of inflammatory cells and immune mediators in progressive CaOx nephropathy leading to CKD is an area of active investigation (discussed further under pathogenesis innate immune response of nephrolithiasis and nephrocalcinosis).

Adrenal Disorders

Nephrocalcinosis and nephrolithiasis have been described in primary hyperaldosteronism due to adenoma producing excess aldosterone leading to significant hypertension and nephrocalcinosis, persisting despite removal of the tumor (406).

Cystic Fibrosis

While some patients with cystic fibrosis (CF) have hypercalciuria, many have hypocalciuria (407). However, several studies have shown an increased incidence of nephrocalcinosis and nephrolithiasis in CF patients (407,408). In 2005, Hoppe et al. (409) demonstrated that absorptive hyperoxaluria owing to the malabsorptive state of CF and hypocitraturia are the main causes of the increased incidence of nephrocalcinosis and nephrolithiasis. Other factors that may influence calcium excretion in CF patients include treatment with diuretics and immobilization.

Renal Tubular Acidosis

Many forms of RTA are associated with an increased incidence of nephrocalcinosis and/or nephrolithiasis usually due to hypercalciuria without hypercalcemia (410). These conditions include primary hereditary disorders causing a defect of the anion exchanger AE1 on intercalated cells of the collecting duct or the distal tubule apical proton pump or H + –K + ATPases (411).

NEPHROLITHIASIS AND CRYSTALLINE NEPHROPATHIES

Clinical Presentation

Renal stone disease (nephrolithiasis) is an important source of morbidity in the United States, and its incidence is increasing worldwide (412,413). Dietary, metabolic, and endocrine factors including obesity, diabetes, hypertension, cardiovascular disease, atherosclerosis, dyslipidemia, and other diseases predispose stone formation. A minority of renal stone disease is hereditary. The majority of patients with nephrocalcinosis are men; most stones in women are due to either metabolic defects such as cystinuria or infection. The peak age at first episode is approximately 30 to 40 years with a later initial presentation

in females (414). Up to 75% of patients will have recurrent stones, sometimes 10 or more over their lifetime (415). Stones are more common in whites compared with nonwhites (416). North American Indians, Hispanics, Blacks in both America and Africa, Asians, and native-born Israelis have fewer stones (415). Stone composition varies somewhat by ethnicity, with calcium oxalate stones being overall the commonest and more common in whites; uric acid, struvite (ammonium magnesium phosphate), and matrix stones are slightly more common in nonwhites (415). The underlying etiology of stone formation is similar, except that whites appear to have a higher incidence of hypercalciuria; the incidence of hypocitraturia, hyperuricosuria, hyperoxaluria, and other metabolic abnormalities are similar among the various ethnic groups studied. Geography and temperature seem to have a relationship to nephrolithiasis in that there is a noticeable increase in urinary calculi in mountainous areas, and there is also a tendency in a given region for the highest incidence of stones to occur in the warmer months (415). These observations suggest that a balance of fluid intake, perspiration, and urinary output is important, and therefore, increasing fluid intake in an attempt to defend against recurrences of renal stones is universally recommended (416). Urinary calculi often make their presence known by an episode of ureteral colic when they become entrapped. There are four typical locations. Stones may become impacted in a renal calyx, at the ureteropelvic junction, at the pelvic brim where the ureter arches over the iliac vessels, or in the posterior pelvis where the ureter is crossed anteriorly by pelvic vessels. Congenital or acquired anomalies of the urinary tract may influence the location of stone formation and passage. Ordinarily, small stones are passed spontaneously. Stones too large to pass remain in the renal pelvis, where they usually continue to grow. Eventually, particularly with struvite or infection stones, they may completely fill the renal pelvis and calyces, creating the staghorn calculus (Fig. 24.75). These stones may create a situation of chronic partial obstruction with hydronephrosis and slowly declining renal function. Often, the situation can be complicated by infection (see under pyelonephritis and reflux). Even stones not due to infection by urea-splitting organisms not infrequently become secondarily infected, as does the overlying kidney. In such instances, there may be sepsis, shock, and precipitous renal failure. The kidney itself shows varying mixtures of acute and chronic pyelonephritis.

The great majority of stones in humans, 70% to 80%, are composed of calcium oxalate or calcium phosphate (Table 24.7). Other calculi are made up of struvite (magnesium ammonium phosphate) in infection stones (10%), uric acid (5% to 10%), cystine (<1%), xanthine (<1%), and more rarely, other substances (417–419). Since treatment varies depending mainly on the type of stone, evaluation of stone composition biochemically is important.

Mechanisms of Stone Formation

Crystal formation and development of calculi can be broken into three interrelated components: (a) urine volume and the degree of saturation of the urine with respect to the crystal system in question; (b) nucleation, or growth of crystals from a seed crystal that in most instances is heterogeneous nucleation—crystal formation on a surface medium—and not homogeneous nucleation (crystal formation in a liquid medium with no surface) and complexation; and (c) the presence and level of inhibitors of stone formation. A brief discussion of each of these major components of stone formation follows.

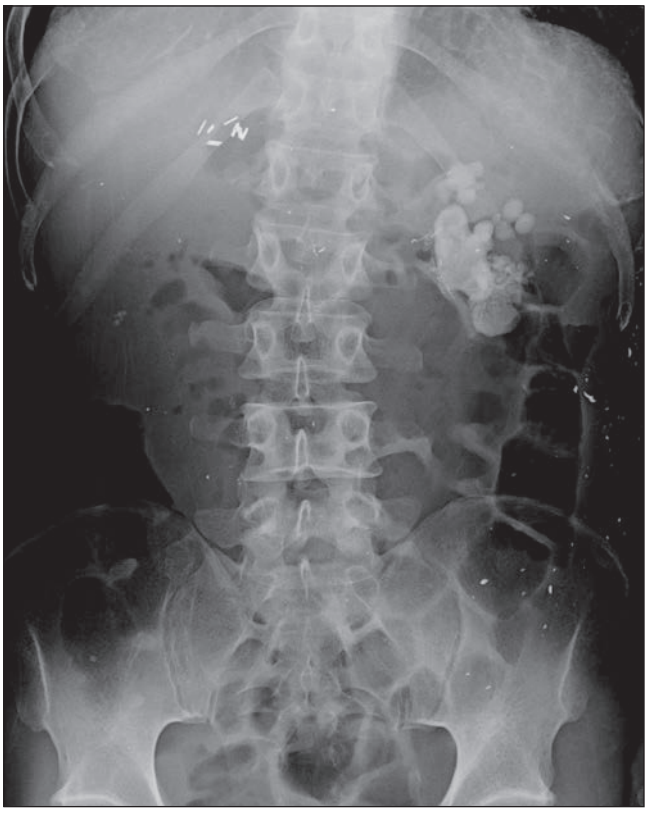


FIGURE 24.75 Staghorn calculus in a 46-year-old man with history of multiple kidney stone extractions. Chemical analysis of the stones showed 100% calcium oxalate monohydrate composition. At the time of this radiographic image, the patient had no pain; he was seen for routine follow-up.

Saturation of Urine

For a stone to form, an absolute prerequisite is that the urine must be supersaturated for the precipitating crystalline phase (420). The state of saturation for any potential crystal system, as, for example, calcium oxalate, depends on several variables, including the concentration of the solutes, pH, and ionic strength. The solubility product of a salt is dependent on the pH of the solution, and the ionic strength, principally from the monovalent ions in the urine, effectively determines the concentration of calcium and oxalate needed to saturate the

TABLE 24.7 Relative frequency of kidney stones

Composition	% of All stones
Calcium, oxalate, and/or phosphate	70–80
Struvite	10
Uric acid	5–10
Cystine	1
Others (xanthine, matrix, medications, etc.)	<1

urine for the crystalline phase. For example, uric acid crystals form in acidic urine; at a pH of 7.0, the solubility of uric acid in urine is good. Acid pH (e.g., 5.0) decreases the solubility of uric acid (421). Normally, approximately 50% of the calcium and approximately 50% of the oxalate in the urine are available in free ionic form (422). By far, the most common type of kidney stones is composed of calcium, for example, calcium oxalate either alone or in combination with calcium phosphate. The term *complexation* refers to soluble complexes with calcium for example oxalate. Complexation effectively reduces the free ion concentration of each. Supersaturation of the urine for salts is a common occurrence, but formation of the solid phase occurs only in certain individuals, suggesting that inhibitory substances are present that modify the ability of crystals to form in a saturated urine, some of which are absent or abnormal in stone formers. Inhibitors of stone formation have been identified and are discussed later.

Nucleation

Heterogeneous nucleation is the common in vivo mechanism for stone formation. This form of nucleation is based on the presence of debris or other crystals that serve as a nidus for development of crystals (422). Heterogeneous nucleation occurs at a lower level of saturation than homogeneous nucleation and therefore requires less energy thermodynamically. Spontaneous, or homogeneous, nucleation is uncommon, principally because of the large expenditure of energy required to initiate it. One example of heterogeneous nucleation is provided by monosodium urate and uric acid, which are excellent heterogeneous nuclei for calcium oxalate (422). Heterogeneous nucleation is thought to form the link between hyperuricosuria and calcium oxalate stones.

Inhibitors of Stone Formation

Normal urine contains chelating agents such as citrate, uropontin, nephrocalcin, THP, glycosaminoglycans, prothrombin F1 peptide, and bikunin (uronic acid–rich protein). These substances exert their effects against stone formation in multiple ways, including primary nucleation, crystal growth, and aggregation (420). Understanding the functions of inhibitors of stone formation has important clinical implications. Some inhibitors, for example, citrate, are clinically used already (423). The main function of citrate is to reduce supersaturation by forming a complex with calcium. Hoyer et al. (424) isolated uropontin, which is a form of osteopontin, from human urine, and subsequently, he and others demonstrated that phosphorylated osteopontin suppresses calcium oxalate crystal growth. In mice deficient in osteopontin, a hyperoxaluric diet resulted in numerous intrarenal calcium oxalate crystals, whereas wild-type mice had no calcium crystals (425). Nephrocalcin, a glycoprotein, has also been isolated from calcium stones and is known to inhibit nucleation, growth, and aggregation of crystals (426). Molecular abnormalities in nephrocalcin have been identified in stone-forming individuals that appear to involve alterations in its phosphorylation and amino acid sequence (426–429).

Aggregation of calcium oxalate and hydroxyapatite crystals can be inhibited in vitro by THP also known as uromodulin. THP is synthesized by epithelial cells in the ascending segment of the loop of Henle and coats their luminal surface. THP has the ability to self aggregate in high ionic strength and, under these conditions, forms part of the stone matrix (429). At low ionic strength, THP is in its unaggregated form and is a potent inhibitor of stone formation by binding calcium oxalate monohydrate; thus, THP inhibits the initial step of microlith formation (see Randal and Evan's theory of microlith formation). THP mutations are causing some forms of autosomal dominant medullary cystic disease and hyperuricemic nephropathy. Therefore, THP gene polymorphisms affecting its structure and ability to bind calcium oxalate monohydrate in the loop of Henle are thought of as a potential cause for stone formation. This is supported by the abnormal handling of uric acid in the kidney found in patients with hyperuricemic nephropathy due to THP (UROM) mutations (see Chapter 4).

CaOx and *Oxalobacter formigenes*

Oxalate has no known intermediary metabolic functions in animals including humans, but is an important source for *Oxalobacter formigenes*, a bacterium in the normal colonic flora in humans (430). There is evidence that enteric colonization of animals and humans with *O. formigenes* is associated with decreased stone formation in the kidney of animals and humans under dietary controlled conditions (431–433). These studies have brought a new understanding of pathophysiology of CaOx stone formation and suggest that changing the microbiotic flora may be key in preventing or treating a disease that is a major public health issue (434).

Chronic CaOx Nephropathy and Innate Immunity

Furthermore, THP is a potent activator of monocytes, and its physiologic function relates to immune regulation (428). Recent studies by Darisipudi et al. (105) showed that crystallized THP in nanoparticle form (Fig. 24.76) is ingested by dendritic cells (DCs) and activates secretion of mature proinflammatory cytokines, including interleukin-1β (IL-1β). IL-1β secretion is mediated by expression by TLRs, TNF-α, or IL-1α and is dependent on NLRP3 inflammasome, its linker molecule ASC,

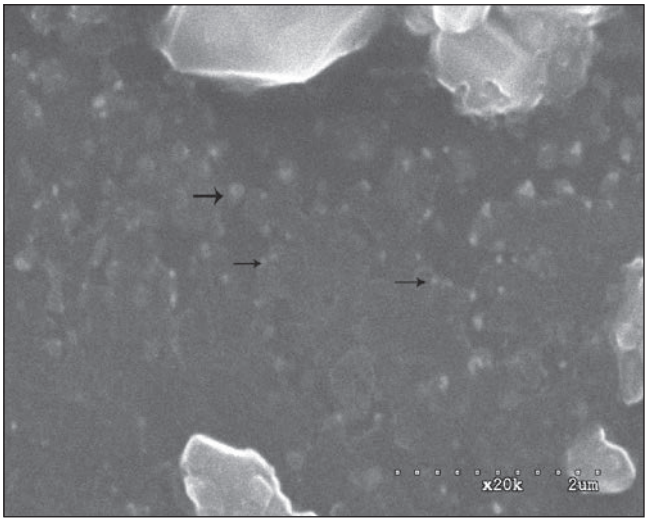


FIGURE 24.76 Tamm-Horsfall protein crystalizes in nanoparticles (*arrows*) that are small enough to be phagocytosed by immune cells. Activated monocytes secrete IL-1β, which mediates innate immunity–mediated inflammatory responses. (scanning electron microscopy × 20k.)

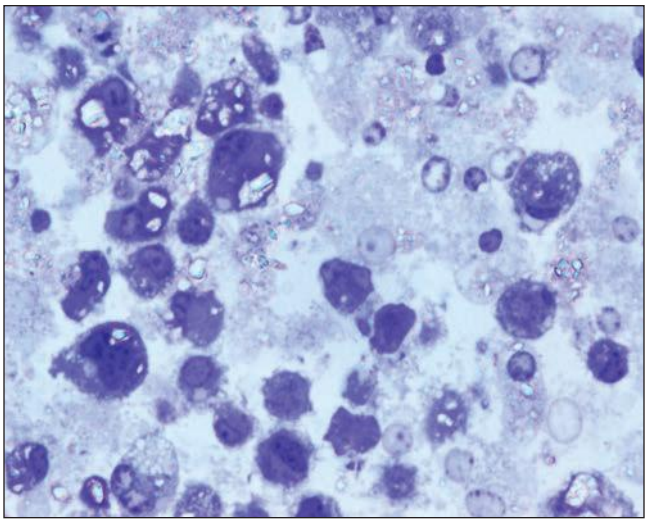


FIGURE 24.77 Murine renal dendritic cells (DCs) grown in culture when exposed to CaOx internalize the crystals, which appear birefringent. IL-1β production follows, through an NLRP3 pathway, thus implicating. (cytospin preparation of cultured DCs × 1000.) (435)

and pro–IL-1β cleavage by caspase-1 (105). Therefore, THP is an NLRP3 agonist handled by antigen-presenting cells as an immunostimulatory nanoparticle (Fig. 24.76). Furthermore, murine renal DCs exposed to CaOx internalize the crystals and become activated to secrete IL-1β through an NLRP3 pathway (Fig. 24.77) (435). These results suggest that NLRP3 inflammasome is involved in the inflammatory response of oxalate nephropathy leading to tubulointerstitial fibrosis and chronic oxalate nephrocalcinosis or stone disease; thus, IL-1β blockade may prevent renal damage in this disease (435). The chronic effects of CaOx deposits and stone formation were independently shown to involve innate immune responses (436).

The link between intestinal microbiota and innate immune response in stone disease is now becoming clearer. CKD affects intestinal reabsorption and secretion of CaOx in more ways than one. For example, metabolic acidosis; increased BUN; volume overload leading to gastrointestinal congestion; iron supplementation, which causes bacteria overgrowth; and vascular calcifications leading to intestinal ischemia are common in CKD patients. Anders et al. (437) suggest that a combination of "*leaky gut*" and altered microbial intestinal flora is intimately related to aberrant innate immune response contributing to CKD complications. Therefore, it is suggested that pre- or probiotics hold therapeutic promise in CKD (437,438).

Another potential calcium oxalate crystallization inhibitor is the F1 fragment of prothrombin (PF1). PF1 is produced by the action of thrombin on prothrombin and is then filtered into urinary filtrate, but can also be synthesized in the kidney. In addition to inhibiting stone formation, PF1 may aid in the removal or detachment of crystals from tubular epithelium (428).

Causes of Calcium Nephrolithiasis

Idiopathic Hypercalciuria

Approximately 95% of idiopathic nephrolithiasis is due to idiopathic hypercalciuria. Patients have a normal serum calcium level but have hypercalciuria, defined as the urinary excretion of more than 300 mg calcium per day in men or more than 250 mg per day in women or more than 4 mg/kg/d. This is not one disease but a condition that affects three organs: the kidney, the bone, and the intestine. A primary defect in one organ (e.g., bone) may lead to symptoms appearing in another organ (e.g., the kidney or intestine) (420). Theories to explain idiopathic hypercalciuria include abnormalities in handling of calcium in the kidney, intestines, or bone leading to an increase in calcium excretion. Some authors believe individual patients may have abnormalities in one specific organ of calcium regulation and have divided patients into absorptive, renal, or resorptive types. Pak et al. (439), examining one group of patients with idiopathic hypercalciuria, found that 44.7% of those with absorptive idiopathic hypercalciuria and 38.5% of those with renal idiopathic hypercalciuria had a family history of stone formation. Recent studies show that familial hypercalciuria is a complex trait determined by genetic polymorphisms and environmental factors similar to hypertension, obesity, and diabetes. Adenylyl cyclase as a possible candidate gene was identified (439,440). Categorization of patients into groups is difficult clinically because of rapid metabolic corrections that offset the initial dysregulation, although the distinction between absorptive and renal hypercalciuria is one worth making in therapeutic terms, since renal hypercalciuria appears to respond to thiazide therapy, whereas absorptive hypercalciuria does not. In addition, in some pedigrees with a severe form of absorptive hypercalciuria, there appears to be a role for the soluble adenylyl cyclase gene, providing a possible future avenue for treatment (439).

The primary therapy in idiopathic hypercalciuria aims at reducing urinary calcium by two approaches, both of which work to decrease supersaturation (348). There is a general agreement that thiazide diuretics are effective in mitigating idiopathic hypercalciuria, reducing recurrent stone formation by approximately 50% over an extended period. Increased fluid intake, such that urine output reaches 2 L/d, is prescribed in combination with diuretic therapy. In addition, limiting protein and sodium intake is generally accepted as a means to decrease calcium excretion (441).

Primary Hyperparathyroidism

Patients with primary hyperparathyroidism have stone disease in >10% of the cases (442,443). Patients with primary hyperparathyroidism typically form calcium oxalate or mixed calcium oxalate-calcium phosphate stones, although the fraction of calcium phosphate in the stones is generally greater in primary hyperparathyroidism than in patients with idiopathic hypercalciuric stone formation.

Chloride Channel Mutations

At least three types of hypercalciuric nephrolithiasis are linked to mutations in the *CLCN5* gene, which codes for the ClC5 chloride channel on the short arm of the X chromosome. X-linked recessive nephrolithiasis, Dent disease, and recessive hypophosphatemic rickets are lumped together under the name *Dent disease complex* (444–447) and share features of low molecular weight proteinuria, hypercalciuria, and other tubular abnormalities leading to Fanconi-type syndromes, and nephrocalcinosis, nephrolithiasis, or both. The ClC5 chloride channel is a voltage-gated channel expressed in proximal tubules, thick ascending limb of Henle loop, and alpha-intercalated

cells of collecting ducts (445). There are several theories for the mechanism by which mutation of the chloride channel results in hypercalciuria and the other abnormalities of Dent disease, most of which are unproven. The ClC5 channel may be important in endocytosis in the proximal tubule, providing a possible explanation for its role in proteinuria. The loss of other electrolytes in the urine by faulty tubular reabsorption may influence handling of calcium by the kidney and allow for hypercalciuria. Other genes may also be responsible in some individuals with features of Dent disease, as demonstrated in a study by Hoopes et al. (446), who reported no mutation in the *CLCN5* gene in 13 patients, suggesting genetic heterogeneity.

The symptoms of Dent disease appear in childhood, with males affected more than females. Renal failure can occur and may be related to the development of nephrocalcinosis. Nonspecific histologic features of tubular atrophy, interstitial fibrosis, and glomerulosclerosis have been reported (443).

Hyperuricosuria

Coe (447) was the first to point out the high incidence of uricosuria, 26.3% overall, among calcium stone formers. In 14.6% of these patients, it arose on its own, in the absence of any sign of hypercalciuria. Other studies find an even higher incidence of hyperuricosuria in calcium oxalate stone formers, up to 41% (446). It has been suggested that uric acid crystals or other urates act as the nidus for heterogeneous nucleation of calcium oxalate (448,449). Regardless of the underlying pathophysiologic factors, the causal association between hyperuricosuria and calcium nephrolithiasis is attested to by the efficacy of allopurinol to limit recurrence of stones in these patients (448). A low purine diet, alkalinization of the urine, and increased fluid intake are also means of reducing uricosuria. Recent advances in genetics and molecular physiology have enhanced the understanding of renal reabsorption and secretion of filtered urate. Most of the genes that affect serum urate level encode urate transporters or associated regulatory proteins (449–452). URAT1 and GLUT9 are the best characterized to date (451). Understanding these transporters is increasingly important as new research unveils their importance in therapeutic choices and genetic association with uric acid levels in humans. New drugs that act specifically on individual renal urate transporters for the treatment of hyperuricemia and gout are becoming a possibility (451).

Primary Hyperoxaluria

This is a rare hereditary deficiency of the liver-specific pyridoxal phosphate–dependent peroxisomal enzyme alanine-glyoxylate aminotransferase (AGT). There are two forms of hereditary hyperoxaluria. Primary hyperoxaluria type 1 (PH1) is caused by a deficiency in alanine-glyoxylate aminotransferase (AGT), leading to decreased conversion of glyoxylate to glycine, allowing excess oxalate synthesis from glyoxylate oxidation. PH2 results from an abnormality in glyoxylate reductase, also leading to increased conversion of glyoxylate to oxalate. Both PH1 and PH2 are autosomal recessive conditions. Numerous mutations in both genes have been identified (453). Aberrant splicing appears to underlie at least some of the defects in PH2 (454). Patients with PH1 tend to have a more severe phenotype and develop nephrocalcinosis, leading to renal failure as well as nephrolithiasis. Stone disease is more common than nephrocalcinosis in PH2; thus, there is typically a milder course.

Primary hyperoxaluria typically makes its appearance in early childhood, in either sex, with recurrent calcium oxalate nephrolithiasis and renal failure related to obstruction, infection, and oxalate deposition within the kidney. In some patients, the disease does not manifest until adult life, and in these patients, the renal disease progresses at a slower pace. The kidney is the only route of excretion of oxalate, so that when renal function declines below the level at which oxalate can be completely filtered (with a small portion excreted by the tubules), oxalate begins to accumulate in the kidney. The kidneys may show widespread deposition of crystals in the tubular lumens, some of which are dilated and others atrophic, as well as in tubular cytoplasm. The crystals can readily be seen by examination under polarized light because of their birefringence (Fig. 24.78).

With the advent of renal failure, deposits of oxalate begin to appear elsewhere in the body, notably in the myocardium (particularly in the conduction system), retina, skin, central nervous system, bone marrow, and blood vessels. With survival extended by dialysis, these deposits pose clinical problems in the form of cardiac arrhythmias, cardiomyopathies, erosive synovitis, digital gangrene, and mononeuritis multiplex, with elevated mortality rates. Combined hepatic and renal transplantation has been used in an attempt to correct both the enzymatic defect and the renal failure, with variable success (455). It is recommended that in patients with primary hyperoxaluria, transplantation be carried out much earlier than in other renal diseases, because oxalate deposits in other organs do not seem to accumulate until renal failure supervenes. About one third of patients are responsive to pharmacologic doses of pyridoxine (vitamin B_6), an enzyme cofactor for the defective enzyme in type 1 hyperoxaluria. Pharmacologic doses attenuate the enzymatic defect in some patients, but its mechanism of action is unknown. Recent studies show that pyridoxine is metabolized to pyridoxal phosphate, the essential cofactor of AGT, increasing enzyme catalytic activity (456).

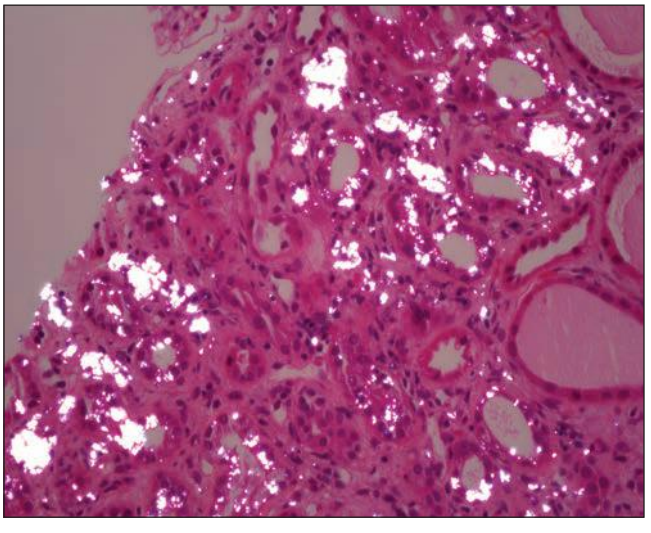


FIGURE 24.78 Recurrent hereditary oxalosis in allograft kidney. There is diffuse CaOx deposition in the tubulointerstitium. (polarized microscopy × 100.)

Secondary Hyperoxaluria

Enteric hyperoxaluria is the most important variant of secondary hyperoxaluria. It is caused by hyperabsorption of oxalate by the gut in various disorders and situations, including Crohn disease, celiac sprue, pancreatic insufficiency, and small intestinal bypass surgery for obesity (457). All of these conditions have in common fat malabsorption with steatorrhea, which increases oxalate absorption from the small intestine and, to a lesser extent, the colon. In the case of jejunoileal bypass, symptomatic stone disease usually appears within 2 years of the operative procedure, requiring lithotomy in about one fifth of patients (458). As mentioned above, oxalate-degrading bacteria such as *O. formigenes* play a role in secondary hyperoxaluria (433).

Other causes of hyperoxaluria are much more rare and include ethylene glycol intoxication, adverse reaction to methoxyflurane, and excess intake of ascorbic acid (vitamin C) or foods rich in oxalate such as cocoa, spinach, beet greens, and rhubarb.

Calcium Oxalate Stones and Randall Plaques

Nearly 80 years ago, Randall using stereomicroscopy of renal papillae disclosed externally visible foci of crystal deposits in 201 of 372 kidneys (54.0%) at autopsy (420,459). Now known as Randall plaques, these lesions are prevalent in patients with oxalate stones and sometimes in patients who do not form stones. Calcifications took the form of linear deposits coursing longitudinally along the papillae and appearing to outline the tubules or larger plaques. Randall plaques were found in 27.9% of cases with kidney stones. Small calculi were found in 30 of 272 cases (8.1%), either attached to or embedded in the side of the papilla. Randall believed that calcium phosphate, calcium oxalate, and uric acid stones began in this manner. Evan et al. (460) shed new light in Randall's observations and showed that CaOx crystals initially form in the loop of Henle, expand in the interstitium encasing the tubules and vasa recta, and eventually, involve the papilla. Biopsies from kidneys of patients undergoing nephrolithotomy or other surgical procedures unrelated to stone disease were examined. The authors found microscopic deposits (as small as 50 nm) almost exclusively along basement membranes of thin limbs of the loop of Henle. These initial sites of crystal deposition were found only in patients who were known idiopathic hypercalciuric CaOx stone formers. No plaques were identified in patients with a history of stones following intestinal bypass or in individuals with no history of stones. The mechanism of action put forward by Randall et al. is considered a milestone; however, other mechanisms seem likely to be operative as well as described under inhibitors of CaOx formation (427–430).

Hypocitraturia

The role of citrate in preventing stone formation is not entirely clear, but it is known that increasing urinary citrate excretion is beneficial, at least in part because citrate complexes calcium and lessens free calcium ion activity (420). Citrate likely acts as an inhibitor of stone formation through decreased crystal growth and aggregation (see Inhibitors of Stone Formation). Hypocitraturia can be caused by stimulation of its reabsorption in renal tubules owing to intracellular acidosis, as is found in distal RTA, high protein diet, chronic diarrhea, or with potassium depletion (461). Administration of alkalinizing agents can correct the acidosis and restore citrate levels in the urine.

Renal Tubular Acidosis

With rare exceptions, stone formation and nephrocalcinosis in RTA are limited to patients with type 1, classic distal RTA. Approximately 75% of patients with type 1 RTA develop nephrocalcinosis, nephrolithiasis, or both (462). Type 1 RTA may be inherited as an autosomal dominant disorder or may occur sporadically without associated disease (463). It may also be acquired in conjunction with other systemic diseases, such as dysproteinemias, Sjögren syndrome, Wilson disease, primary biliary cirrhosis, lymphocytic thyroiditis, and intestinal bypass surgery, as well as with the use of certain drugs, including amphotericin B, lithium, and toluene (463). Carbonic anhydrase inhibitors, which produce a clinical picture of RTA type 2, paradoxically result in stone formation (464).

Patients with RTA have renal tubular cell acidosis, which results in increased calcium and phosphorus excretion, decreased citrate excretion, and a high urine pH, all of which are conditions that raise the likelihood of crystal formation. Therapy consists of alkali administration to acidify the urine, reduce calcium excretion, and increase urinary citrate, slowing the pace of nephrolithiasis and nephrocalcinosis. Treatment with potassium citrate tablets has been shown to be effective and is better tolerated than sodium bicarbonate (465).

Non Calcium Stones

Uric Acid Stones

About 5% to 10% of renal stones are uric acid stones (Fig. 24.79). There is a higher incidence of uric acid stone formation among patients with gout; from 10% to 50% form uric acid stones, the variation being a result of differences in rate of uric acid excretion (466,467).

Uric acid crystals develop in urine of low pH, especially when the uric acid content is high (420). However, low urine pH and low urine volume are more important than the absolute content of uric acid in the development of stones in patients with gout as well as those with idiopathic uric acid stones. Idiopathic uric acid stone formers tend to excrete less ammonia, leading to the low urine pH values found. Those patients with gout who form stones also tend to have low

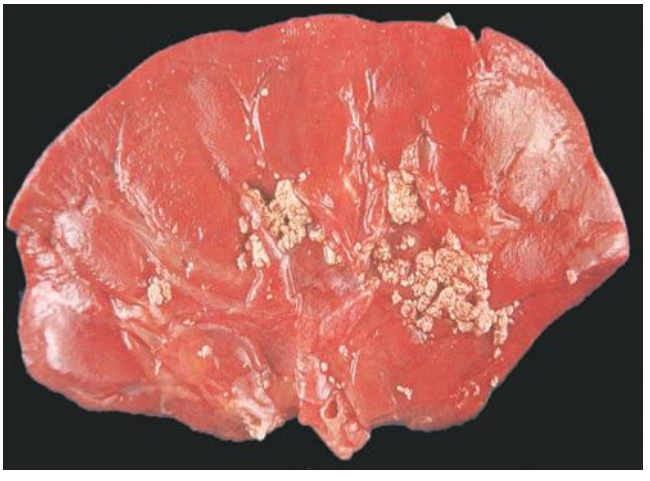


FIGURE 24.79 Uric acid nephrolithiasis. Multiple small pale yellow uric acid stones in the medullary zone.

urinary pH values, suggesting a defect in ammonia production in this group as well (468). The higher prevalence of uric acid stones in some countries, such as the middle eastern countries (40% or more), testifies to the role of dehydration in formation of these type of stones (420,450).

Low-carbohydrate, high-protein diets also tend to reduce urine pH, increasing the risk for urolithiasis (469). A connection between insulin resistance and uric acid nephrolithiasis has been suggested, since individuals with gout share features of the metabolic syndrome, and there is a higher incidence of uric acid stone formation in type 2 diabetic stone formers (470). Insulin resistance is also associated with an acidic urine (471), the condition most favorable for uric acid stone formation.

A smaller group of uric acid stone formers consists of those patients in whom there is overproduction of uric acid. The simplest examples of overproduction of uric acid are malignancies, primarily lymphoproliferative and myeloproliferative disorders, in which increased nucleotide catabolism generates excess purines and hence excess uric acid. Chemotherapy, with abrupt tumor necrosis, may lead to the same result, with stone formation and even obstruction (472). Polycythemia, hemolytic anemia, and sickle cell disease can all increase uric acid formation. Enzyme defects, all of which are rare, may also lead to hyperuricemia. These conditions include hypoxanthine-guanine phosphoribosyltransferase deficiency (Lesch-Nyhan syndrome), adenine phosphoribosyltransferase deficiency, elevated phosphoribosylpyrophosphate synthetase activity, decreased phosphoribosylpyrophosphate substrate utilization, and type 1 glycogen storage disease. Simple urine alkalinization can be therapeutic in some of these conditions (473).

Gouty microtophi can be identified in individuals with clinical symptoms of gout. In renal biopsies, uric acid crystals are seen as clear "needles" within the interstitium, usually surrounded by an inflammatory reaction that can contain giant cells (Fig. 24.80). Uric acid crystals tend to dissolve in formalin fixed tissue and are better seen in unfixed specimens under polarized light or stained with Gomori methenamine silver.

Cystine Stones

Cystinuria is a hereditary disorder of tubular transport, which results in the excretion of abnormally large amounts of cystine, as well as other dibasic amino acids, in the urine and ultimately the development of recurrent cystine stones (474). The disease is inherited in most cases as an autosomal recessive trait (type 1) or incomplete recessive (types 2 and 3). The incidence varies from 1 in 2500 to 1 in 15,000 (474). Renal stones typically begin to form in the second or third decade, but they may appear earlier or later. Cystine stones constitute approximately 2% of all urinary stones. The typical hexagonal crystals are usually readily recognized in the urine, particularly a concentrated early-morning urine.

The genetic defect in type 1 is a mutation in the gene SLC3A1 (solute-linked carrier 3A1), whereas types 2 and 3 are associated with mutations in SLC7A9. These genes code for cystine, dibasic, and amino acid transporters (474–476). The renal calculi that form may appear as multiple small stones or large staghorn calculi; radiographically, they can be of two types, with either a smooth or a rough surface. Grossly, they are granular and often sand colored to yellow brown. Cystine may be admixed with oxalate, phosphate, and struvite in some patients. The risk in these patients, as with other forms of nephrolithiasis, is secondary infection and obstruction.

Cystinuria should be distinguished from *cystinosis*. In that condition, also a rare hereditary enzymatic disorder, there is widespread *intracellular* accumulation of cystine, with extensive damage, particularly in the kidney, and ensuing renal failure. Cystine crystals are rectangular or needle shaped mostly within macrophages but sometimes in tubular epithelial cells or podocytes. A helpful clue in cystinosis is multinucleated podocytes, a finding rare in other diseases (477). Atubular glomeruli and the so-called swan-neck deformity in proximal tubules are also found in cystinosis but are nonspecific findings (478).

Miscellaneous Stones

Xanthinuria

Xanthinuria is a rare hereditary disorder, transmitted as an autosomal recessive trait (479). There is a deficiency of

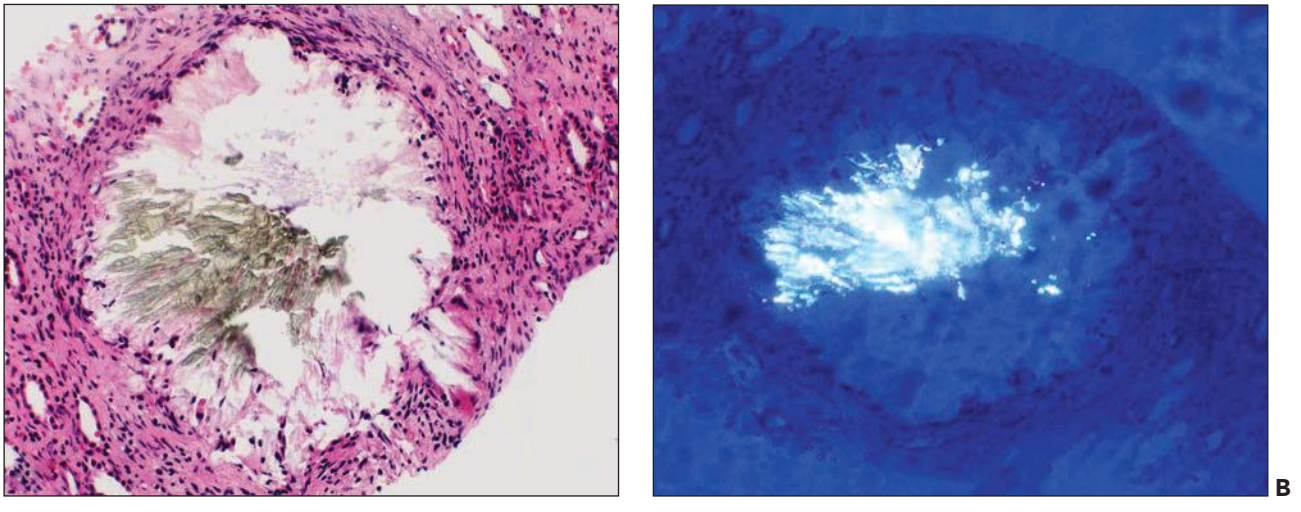


FIGURE 24.80 Gouty tophus in renal biopsy from man with stage IV kidney failure of unknown etiology. A: Uric acid crystals in the medulla are surrounded by inflammatory cells. (H&E; ×400.) **B:** Uric acid crystals are birefringent under polarized light.

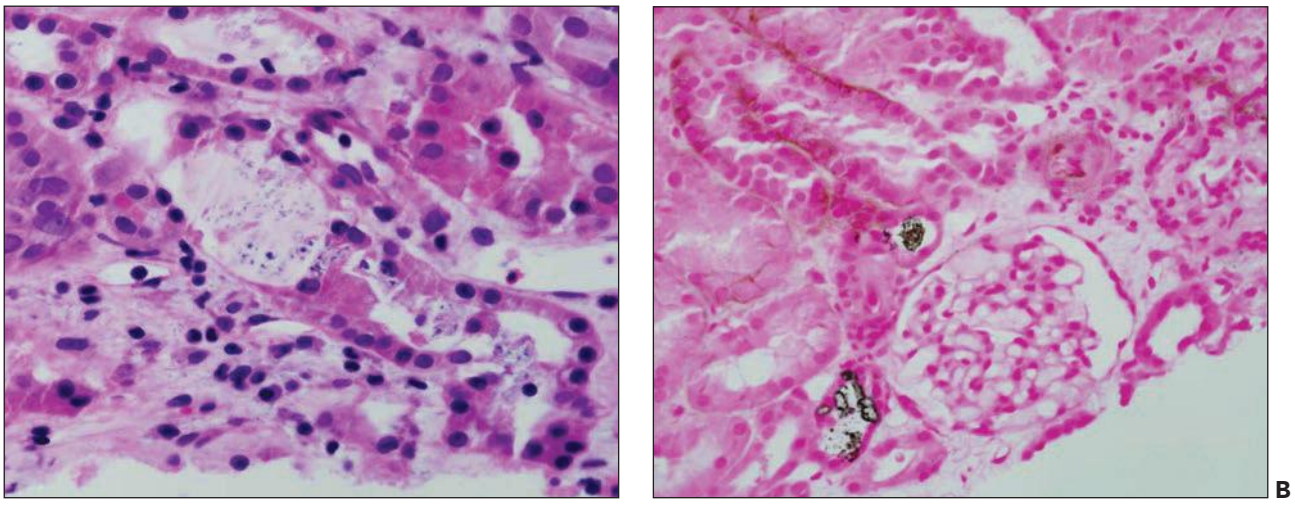


FIGURE 24.81 Foscarnet nephropathy. Renal allograft biopsy from a child treated for drug-resistant cytomegalovirus infection. **A:** Deposits are barely visible on H + E. **B:**They stain black with von Kossa stain (×400.)

xanthine dehydrogenase, an enzyme responsible for the oxidation of hypoxanthine and xanthine to uric acid, such that xanthine and hypoxanthine appear in the urine in greatly increased quantities. Conversely, serum urate and urinary uric acid levels are very low. Radiolucent xanthine stones are found in about one third of patients with this disorder.

2,8-Dihydroxyadeninuria Deficiency

This is a rare disorder inherited by autosomal recessive mode that leads to the formation and hyperexcretion of 2,8-dihydroxyadenine (DHA) into urine (480). DHA is insoluble in urine in the normal range of PH and may lead to crystal formation in the urine and kidney. Patients present with AKI and, in severe cases, progress to CKD. The crystals accumulate in tubular epithelial cells and the interstitium, appear brown green on H + E, and are strongly birefringent under polarized light. DHA crystals are fan-like, are rhomboid or needle-like, and resemble oxalate crystals. The best method for accurate diagnosis is X-ray microanalysis. DHA crystals are devoid of calcium in contrast to oxalate crystals, which usually complex with calcium (477,480).

Medication-Related Stones

Precipitation of exogenously administered medications or crystals induced by certain drugs can cause acute and chronic kidney injury and crystal nephropathy. Risk factors include preexisting conditions such as hypovolemia, underlying kidney disease, and metabolic disorders that promote changes in urinary pH favoring crystal precipitation. Sulfadiazine; acyclovir foscarnet, a phosphoric acid derivative used for CMV and herpes viruses resistant to acyclovir or ganciclovir; indinavir; triamterene; and methotrexate are known to cause crystal nephropathy. Orlistat, ciprofloxacin, and oral sodium phosphate solution have been added to the list in the last decades (481). Foscarnet crystals are clear on H + E; they stain black with von Kossa stain because of admixed sodium calcium salts (Fig. 24.81). Triamterene is a weak diuretic used to preserve potassium and prevent hypokalemia and can be complicated with crystal deposits in renal tubules and the interstitium (481). In H + E, the crystals appear yellow, and they are strongly birefringent. Maltese cross profiles are characteristic. The appearance is similar to DHA crystals (477,480).

Treatment of Stones by Lithotripsy

Most stones will pass spontaneously and do not require intervention (482). Stones that are 4 to 5 mm have a 40% to 50% chance of spontaneously passing in the urine. For those stones that are >5 mm, spontaneous passage is less likely, and further intervention is necessary. Stones in the middle to distal ureter have a greater chance of passage than those in the proximal ureter or calyces. Shock wave lithotripsy (ESWL) has revolutionized the management of urinary calculi although for distal calculi, some authors recommend ureteroscopy over SWL for

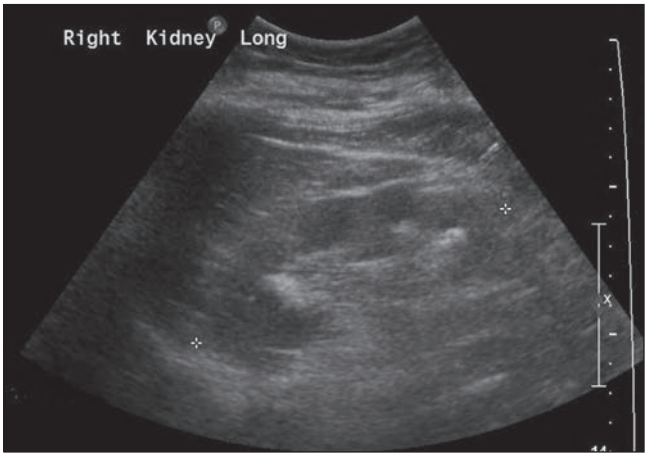


FIGURE 24.82 Nephrolithiasis. Sonogram shows increased renal echogenicity compatible with renal parenchymal disease. Echogenic foci in the lower pole of the right kidney represent small nonobstructive stones. The patient was a 64-year-old woman with previously diagnosed medullary nephrocalcinosis who presented with elevated creatinine.

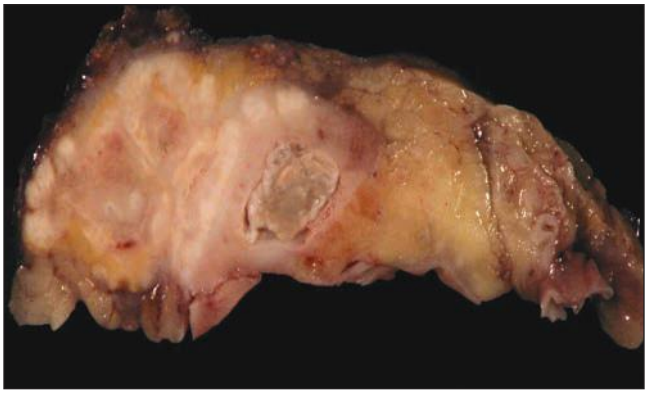


FIGURE 24.83 Nephrectomy for kidney stones and repeated infections shows impacted stone and atrophic renal parenchyma. The patient was a 67-year-old man with a history of staghorn calculi and end-stage kidney disease.

the overall higher success rate and reduced need for secondary interventions or retreatment (483,484).

The lithotripter technology focused acoustic waves on the stone, such that the stone disintegrates as a result of erosion at the points of entry and exit of the shock waves. The overall stone-free rate at 3 months following ESWL is nearly 75%. With stones smaller than 1.5 cm, the stone-free rate is approximately 90%. The method has advanced to use lasers instead of acoustic waves (484). Most stones smaller than 2 cm will be successfully removed by lithotripsy. Larger stones (Figs. 24.82 and 24.83) may be impacted and cause severe injury and end-stage kidney failure (Fig. 24.84). Ureterostomy or percutaneous nephrolithotomy is an alternative treatment for larger stones (485). In the setting of urinary tract anomalies, complex stones or those in the distal ureter, or in select groups of patients such as children or the obese, treatment should be individualized depending on stone size, location, and the particular circumstances (486). Cystine, calcium oxalate monohydrate, and matrix stones are more refractory to treatment by lithotripsy than are other stones, such as those formed from uric acid, and may require ureterostomy or percutaneous nephrolithotomy.

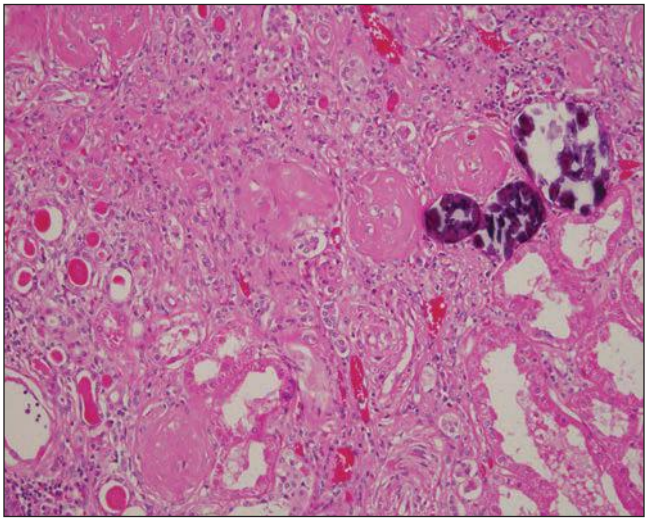


FIGURE 24.84 End-stage kidney disease secondary to nephrolithiasis and repeated urinary tract infections from a 46-year-old man with bilateral nephrolithiasis. Sections show globally sclerosed glomeruli, tubulointerstitial atrophy, and fibrosis; focal calcium phosphate deposits and tubule thyroidization are also apparent. (H&E; ×400.)

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