

Non-proliferative Lesions of the Kidney and Lower Urinary Tract in Rats

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INTRODUCTION

The purpose of this guide is to present a standardized classification of the non-proliferative lesions of the urinary system that can be readily used by the toxicologic pathologist. The urinary system in all mammalian species, including the rat, is represented by the kidney and lower urinary tract. The lower urinary tract is comprised of the ureters, urinary bladder and urethra. In addition to excretion of the waste products of metabolism, the functions of the kidney include elaboration of hormones, selected metabolic activities, and body homeostasis such as control of water volume, electrolyte concentration, and low-molecular-weight protein turnover. The primary function of the lower urinary tract is the transport of urine from the renal pelvis through the ureters to the urinary bladder where it is stored until eliminated. The urethra serves as a conduit for excretion of urine from the urinary bladder to the exterior.

The kidneys of the rat are located retroperitoneally, ventrolateral to the vertebral column, with the right kidney positioned cranially within the protective province of the

rib cage. The mature kidney is bean-shaped with a single papilla surrounded by the renal pelvis, which represents an anterior dilation of the ureter. Depending on age and gender, each kidney weighs approximately 0.51 to 1.08% (mean 0.65%) of the total body weight (1). From the standpoint of toxicologic pathology, the important elements of the kidney are the nephron subunits (glomerulus, proximal tubule, descending and ascending limbs of Henle, distal convoluted tubule), and the collecting duct system, interstitium and juxtaglomerular apparatus. In the rat, the proximal tubule can be subdivided into P1 and P2 segments (representing the convoluted portion) and the P3 segment (representing the pars recta or straight segment). Proceeding from the outer to the innermost surface, the rat kidney parenchyma is divisible into 5 zones, namely the cortex (containing glomeruli), outer stripe of the outer medulla (containing pars recta), inner stripe of the outer medulla, inner medulla, and papilla. Wherever possible, toxic renal responses should be classified and recorded on the basis of structure (e.g. glomeruli, tubule segment or interstitium), and topographical location (zones involved in toxicity). Glomerular change can be described as diffuse when it affects all glomeruli, or focal/multifocal when involving only some. Within each glomerulus, the change can be global, affecting the whole corpuscle, or

segmental, involving only some intraglomerular tufts. An axial pattern refers to an arborizing disposition within the corpuscle. Likewise, kidney tubule changes can be qualified as diffuse, focal/multifocal, or solitary. Lesions may be segmental if they follow the radial distribution of the nephrons, or zonal if restricted to a specific zone.

Xenobiotic-associated kidney injury typically depends on selective concentration of a toxic moiety in the nephron as a result of the organ's normal physiologic function. The kidney also has the enzymatic capacity to metabolize some xenobiotics to reactive intermediates. The manifestation and pattern of induced renal injury are dependent on the inciting xenobiotic and its mode of action. Although degenerative changes or cell death may affect any part of the nephron or ducts, a primary consideration in renal toxicologic pathology is the propensity for the nephron to respond to injury often as a unit rather than only at the site of injury.

Each of the 3 segments of the lower urinary tract in rats are lined by transitional epithelium, commonly referred to as urothelium, representing the main metabolically active cell population. Since the entire lower urinary tract is lined by transitional epithelium that is surrounded in turn by both a subepithelial connective tissue and a muscle coat, the ureters, urinary bladder and urethra all have the potential to develop similar non-proliferative lesions. Under normal conditions, the urothelial lining of the bladder has a very low mitotic index post-weaning (3). However, because of its storage function, the bladder is susceptible to injury and the lining cells readily respond to stimuli such as crystals, calculi and chemicals within the urine. In contrast, spontaneous or induced ureteral and urethral non-proliferative lesions are rare in the rat. The reason for this difference in lesion frequency between regions may be related to the rapid passage of urine through these ducts compared to the lengthy contact time occurring in the bladder. While proliferative lesions of the lower urinary tract most commonly involve the transitional epithelium, non-proliferative lesions can involve all layers; that is the transitional epithelium, the subepithelial connective tissue, and the muscle coat.

KIDNEY

The diagnostic terms that follow are grouped according to whether they are congenital changes, disturbances of cell growth/differentiation, degenerative changes, cell death, and inflammatory, vascular or miscellaneous changes. In addition, several pathologic conditions specific to the kidney are described under special disease processes.

CONGENITAL CHANGES

The majority of congenital renal changes have been

described as rare, sporadic events, although in certain strains heritable events have occurred at a relatively high incidence (8). The most commonly encountered congenital lesion in outbred strains of rat is hydronephrosis.

Agensis

The occasional absence of one kidney as a congenital change has been observed in many strains of rat.

Hypoplasia (Figure 1)

Renal hypoplasia is usually unilateral, and may affect kidneys in a diffuse or segmental manner. Affected kidneys are reduced in size, weight and volume. Histologically, along with the effects of reduced size on tubule number, cortical fibrosis with dilated/cystic tubules may be present, most prominently along medullary rays. Hypoplasia of renal vessels is also evident. This condition appears to have a genetic basis in Wistar rats as well as in crosses of Wistars with hooded varieties (25).

Polycystic kidney (Figure 2)

Polycystic kidney disease can occur as either a congenital or acquired disorder, representing progressive distension of tubule lumens and glomerular capsules. The condition is usually bilateral, characterized by diffuse symmetrical renal enlargement. There are radially arranged dilated and cystic tubules in both the cortex and medulla, but casts are usually absent. Interstitial fibrosis and atrophy of non-cystic cortical tubules may be present. While this condition has a genetic basis in several species, in the rat it is sporadic and rare except in the MRC/H strain. In the MRC/H strain, it has been determined that distal tubules and ascending portions of the loops of Henle represent the altered polycystic structures (39). Experimentally, the disease may be induced rapidly in rats with diphenylthiazole, producing saccular and fusiform dilation of all collecting tubules in the outer medulla with occasional distension of cortical collecting tubules and distal tubules (10).

Adrenal rest (Figure 3)

This rare anomaly is characterized by the presence of a small aggregation of adrenal cortical cells either attached to the exterior of, or immediately beneath, the renal capsule. The cell aggregate is surrounded by a thin fibrous capsule.

Hydronephrosis

Congenital hydronephrosis occurs occasionally in most strains of laboratory rats, and occurs quite frequently in some albino strains. It can also be an acquired disorder. Hydronephrosis is characterized by dilation, to varying degrees, of the renal pelvis and calyces, and it is usually progressive. It is often unilateral and the right kidney is affected more commonly than the left in most strains of rat

(40). Mild hydronephrosis may not be detected grossly until the affected kidney is sectioned sagittally or transversely. Microscopically in the mildest form of hydronephrosis, the renal papilla can be compressed without any apparent alterations in the urothelium, interstitium, tubules, glomeruli and blood vessels. Although the only consistent external observation may be an increase in the intrarenal diameter (16), moderate hydronephrosis is associated with loss of medullary and papillary tissue due to pelvocalyceal dilation. In kidneys severely affected with congenital hydronephrosis, a spectrum of changes have been described in various strains (31, 33, 34). These include: decrease in number of glomeruli, mild dilation of collecting tubules, mild focal interstitial fibrosis, mineralization and focal segmental glomerulosclerosis. Inconsistent findings include hemosiderin deposits and calcium concretions in the papilla, and calculi in the calyces, changes that are more commonly seen in acquired hydronephrosis. In the most severe cases, the kidney becomes a fluid-filled sac lined by thin attenuated urothelium, perhaps with multilocular cavities, subtending a small rim of atrophic renal cortex (31).

Hydronephrosis may be acquired as a secondary complication of demonstrable obstruction of the urinary outflow at any point from the pelvic hilus to the urethra, for example, as a result of chronic inflammation of the lower urinary tract. However, in congenital hydronephrosis there is usually neither gross nor histological evidence of obstruction of the urinary tract, although in ACI/N rats with hydronephrosis, direct communication of the ureter of the affected kidney with the spermatic canal has been demonstrated (19). It has also been suggested (50), but not confirmed (46), that obstruction of the right ureter by the right spermatic or ovarian artery accounted for unilateral congenital hydronephrosis. More recently, it was shown that, in comparison to normal kidneys, hydronephrotic kidneys have higher pressure in the pelvis than in the ureter due to partial functional obstruction at the ureteropelvic junction (15).

DISTURBANCES OF CELL GROWTH/ DIFFERENTIATION

Glomerular Atrophy (Figure 4)

Glomerular atrophy is characterized by the shrinkage and contraction of glomerular tufts, often with a corresponding enlargement of Bowman's space. It is seen in advanced stages of age-related (spontaneous) chronic progressive nephropathy (CPN) and chronic infarction.

Tubule Atrophy (Figures 5)

Tubule atrophy, a feature of chronic infarction, is represented by contraction and collapse of tubular structures, ultimately with disappearance and reduction of individual tubules. It may be focal, multifocal, segmental or zonal, and usually occurs in a radial or pyramidal

pattern. Affected tubules display condensed epithelium with reduction or obliteration of the lumen, and with markedly thickened basement membrane. There may be a varying degree of peritubular fibrosis and mild interstitial mononuclear cell infiltration. Adjacent, non-atrophic tubules may display lining cell hyperplasia or basophilia.

Cell/Tubule Hypertrophy (Figure 6)

On occasion, when increased kidney weights are recorded gravimetrically, renal tubule hypertrophy may be the only microscopic finding. Morphologically, hypertrophy is characterized by an increase in cell size, but not cell number, involving the epithelial lining of renal tubules. Hypertrophic tubules show a single layer of tall cuboidal to columnar cells with broad bases. The apical border of affected cells may be rounded, projecting into the tubule lumen. The nuclei are often located in an apical position and nucleoli may be prominent. The cytoplasm of hypertrophic tubule cells is often brightly eosinophilic, but it can be pale, chromophobic or oncocytic (20). Tubule cell hypertrophy may be a spontaneous, age-related phenomenon, occurring during the second half of the lifespan in most strains (51), or alternatively its incidence and severity may be increased after treatment with drugs and chemicals (8). For example, distal tubule hypertrophy can develop after proximal tubule cell necrosis as a compensatory response to transient loss of proximal tubule function (44). Proximal tubule cell hypertrophy is also observed in surviving islands of otherwise normal parenchyma in advanced cases of CPN.

Tubule Regeneration (Figure 7)

The regenerative response results in replacement of lost cells, restoring a damaged tubule to its normal cellular state usually with no overproduction of cells (30). Thus, renal tubule cell regeneration is classically exemplified by the epithelial response following coagulative necrosis induced by certain acutely-acting nephrotoxins and may be restricted to specific segments of the nephron. During restoration of a normal tubule, regenerating cells may show a transition from flattened to low cuboidal forms, but the cell number or tubule size is not usually increased. Regenerating epithelium is characterized by basophilia and, temporarily, a higher than normal proliferative rate demonstrable by increased mitotic figures (1, 30). If the injurious insult is chronically sustained, the regenerative process will also be prolonged. In long-standing, persistent tubule regeneration there is often an overproduction of cells in the form of simple tubule hyperplasia. Tubule regeneration induced by xenobiotics should be distinguished from the basophilic tubules characterizing early CPN (30). It should also be distinguished from basophilic tubules observed in the absence of any preceding acute cellular injury.

Karyomegaly/Karyocytomegaly/Multinucleation

Karyomegaly (conspicuous increase in nuclear size), karyocytomegaly (increase in cell and nuclear size), and multinucleation (presence of multiple nuclei in a single cell) are most frequently seen in tubule epithelium with the nuclear change being the most prominent feature. DNA replication without completion of the mitotic process of cytokinesis leads to karyomegaly or multinucleation and is indicative of increased ploidy levels. The pathogenesis is not known but many agents that cause karyomegaly also inhibit mitosis. These changes can occur in different segments of the tubules and ducts, and also in the urothelium. Karyomegalic cells have abnormally enlarged vesicular nuclei that can be up to 8 to 10 times the size of normal nuclei. Affected nuclei may exhibit hyperchromatic (basophilic) staining, irregular outline, multiple nucleoli, and sometimes pseudoinclusions. The enlarged cells appear to bulge into the tubule lumen. They are usually solitary but may occasionally occur in groups.

Karyomegaly is often encountered in the rat, but multinucleation is rare. The presence of karyomegalic epithelial cells in the kidney generally implies a response to a variety of toxic agents, such as proteins, alkaloids, and heavy metals, some of which are renal carcinogens (26,47,57). The location of karyomegalic epithelial cells is dependent on the toxic agent. For example, in lysinoalanine toxicity, karyomegalic cells are limited to the pars recta of proximal tubules (47). Aflatoxin B₁ produces effects mainly in the loop of Henle whereas heavy metals are more likely to cause karyomegaly in the renal cortex (42). Although induced by renal carcinogens, karyomegaly is not regarded as a preneoplastic lesion since there is no evidence that affected cells contribute to the initial formation of proliferative foci (30).

Bowman's Capsule Metaplasia/Hyperplasia

The single cell layer of the parietal epithelium of Bowman's capsule can undergo a metaplastic change from the normal squamous epithelium to cuboidal cells resembling those of proximal tubules. At the same time, there is an increase in the number of cells, representing hyperplasia (30). Metaplasia may occur spontaneously in older rats, but more commonly in males than females. It has also been recorded in spontaneously hypertensive rats (21, 24).

Squamous cell metaplasia

In the rat kidney, squamous cell metaplasia involves the transitional epithelium of the renal pelvis. Histologically, the transitional epithelium shows a focal, multifocal or diffuse replacement by squamous cells. The cellular axes of metaplastic squamous cells lie parallel to the basement membrane and the nuclei are mostly oval or flattened. The surface of the metaplastic squamous epithelium can be either non-keratinized, highly keratinized, or

contain only keratohyaline granules. When present, keratinization can be extensive and the cornified material desquamated. Squamous cell metaplasia of the pelvic urothelium may be accompanied by hyperplasia, and sometimes there is cellular atypia and nuclear pleomorphism. Squamous cell metaplasia may occur as a response to the administration of nephrotoxicants, vitamin A deficiency, or chronic inflammation.

Osseous Metaplasia (Figure 8)

Osseous metaplasia, unrelated to renal mineralization has been observed in the kidneys of Fischer F344 rats (42). In this strain the osseous metaplasia has occurred focally within the interstitium of the renal cortex with displacement of parenchyma. The boundary with adjacent cortical tissue is usually very irregular.

DEGENERATIVE CHANGES

Vacuolation (Figures 9-10)

Vacuolation can involve the cells of any part of the nephron, collecting system, or urothelium, but is most frequently seen in the proximal convoluted tubule. It is recognized as clear, round spaces of variable size within the cytoplasm. Vacuolation may be a transient physiologic response, for example to sucrose in the diet, or it may represent an initial stage preceding cell degeneration (1, 42). Clear vacuolation of variable dimensions suggests hydropic change, typically of phagolysosomal origin. Smaller, more uniform translucent vacuoles suggest fat or lipoprotein accumulation, as in a lipidosis such as aminoglycoside phospholipidosis. Phospholipid accumulation in potassium deficiency is manifest as vacuolation in collecting ducts of the inner medulla.

Hyaline Droplets (Figures 11-12)

Hyaline droplets are eosinophilic cytoplasmic bodies of variable size occurring predominantly in proximal convoluted tubules, sometimes extending into the outer stripe to involve the pars recta. They represent enlarged secondary lysosomes containing protein. Their profile is usually circular but when composed of essentially pure protein of a specific type the droplets may assume a crystalloid or polyangular configuration. Hyaline droplets occur in small numbers in the P2 segment of proximal tubules in the normal mature male rat, where predominantly they represent reabsorption of the poorly catabolized protein, alpha_{2U}-globulin (α_{2U}-g). This low-molecular-weight protein is one of the most abundantly synthesized proteins in male rat liver. Excessive hyaline droplet accumulation in proximal tubules generically termed *hyaline droplet nephropathy*, occurs under conditions that lead to protein overload in the tubules. Potentially, the protein can be any of those normally catabolized by the kidney. Two well-defined examples include the unique

nephropathy induced by a diverse range of xenobiotics exclusively in the male rat, and the renal protein overload associated with generalized histiocytic sarcoma in both males and females. In the xenobiotic-induced nephropathy, the accumulating protein is α_{2U} -g (9) and the condition is specified as α_{2U} -g nephropathy. In histiocytic sarcoma, the protein is lysozyme synthesized and released into the circulation by the neoplastic mononuclear phagocytes (27). The visualization of hyaline droplets is not always easy in sections stained conventionally with hematoxylin and eosin (H&E), but is considerably enhanced with Mallory Heidenhain stain.

Inclusion Bodies (Figure 13)

Inclusion bodies are rounded intracellular profiles of homogeneous appearance. They should be specified as either intracytoplasmic or intranuclear.

Intracytoplasmic Inclusions

Intracytoplasmic inclusions are small eosinophilic or basophilic bodies, other than hyaline droplets or pigment deposits, that are restricted to the cytoplasm. Structurally, intracytoplasmic inclusions may represent myeloid bodies, giant mitochondria, or proliferation of smooth endoplasmic reticulum, usually involving proximal tubule epithelium. Myeloid bodies occur in the cytoplasm of proximal tubule cells after gentamicin administration, where they represent enlarged lysosomes containing myelin figures (45). Amphiphilic cationic substances have also induced myeloid bodies in tubule cells. Proliferation of smooth endoplasmic reticulum has been seen with a number of compounds, sometimes in association with an induction of microsomal enzymes (45). Pleomorphic inclusions representing atypical mitochondria have been observed in oncocytes induced by lead hydroxide (4).

Intranuclear Inclusions (Figure 13)

Intranuclear inclusions can be eosinophilic or basophilic and may occupy much of the nuclear volume. The best known example is the eosinophilic, acid-fast, intranuclear inclusion associated with exposure to lead salts, but these lesions have also been induced by novel pharmaceuticals (20).

Pigmentation (Figure 14)

In H&E sections, renal pigmentation is characterized by intracytoplasmic accumulations of yellow to brown stippling or granules, most commonly seen in proximal convoluted tubules. Renal pigmentation is exacerbated by hepatic dysfunction, hemolysis and other blood-related diseases such as large granular cell lymphoma, where there is an immune-mediated hemolytic anemia. The most commonly encountered pigments are hemosiderin, lipofuscin or bilirubin. Special stains are required for specific differentiation of each pigment type. Thus iron-contain-

ing pigment stains blue with Perl's stain (the Prussian blue reaction); lipofuscin stains blue with Schmorl's method, green with Nile green A, and is usually sudanophilic, iron-negative, and acid-fast; and bilirubin stains green with Hall's method. Some degree of renal pigmentation is present in most aging rats, and diagnosis of compound-related abnormality should be based upon levels of pigmentation in companion controls.

Tubule Dilation (Figures 15-16)

Tubule dilation may be focal, multifocal or diffuse. It is subcategorized into simple and cystic forms.

Simple Tubule Dilation (Figure 15)

In simple tubule dilation, affected tubules show a mild to moderate increase in lumen diameter, but the epithelial lining usually remains relatively normal in appearance. The distribution can be radial or zonal. Simple tubule dilation may be induced by administration of steroids, temporary periods of ischemia, luminal obstruction by crystalline deposits, hyperplasia, inflammation, or tubule cell degeneration/regeneration (20, 22).

Cystic Tubule (Figure 16)

Cystic tubules represent marked focal distensions developing from any segment of the nephron, including Bowman's space. They are usually cortical lesions, but are occasionally observed in the medulla. They may be solitary or multiple, and are either microscopic in size or visible grossly as shiny circular or ovoid structures that bulge through the renal capsule. Microscopically, the cyst cavity is usually lined by a single layer of very flattened epithelium, but larger lesions may also have a thin fibrous capsule. The cyst lumen may be empty or contain material of variable staining intensity representing proteinaceous, cellular or necrotic debris.

Causative factors are poorly understood, but cystic tubules may develop secondarily to tubule obstruction by casts or interstitial fibrosis. Thus, cysts are a common morphologic feature of advanced stages of CPN. Experimentally, cystic tubules can be induced by such compounds as angiotensin-converting-enzyme (ACE) inhibitors or high doses of diuretics. Because electrolyte and fluid volume replacement alleviates the renal changes, this phenomenon is presumed to be secondary to excessive electrolyte loss rather than representing toxic tubule damage (22).

Tubular Cast (Figures 17-18)

Tubular casts are uniform inclusions occupying a length or cross section of a tubule lumen. They should be subcategorized as either hyaline or granular. Casts of both types tend to cause distension of the affected tubule.

Hyaline Cast (Figure 17)

Hyaline casts represent contents of a homogeneous

and eosinophilic nature, which fill the tubule lumen. Hyaline casts are typically composed of protein. They are associated with increased protein in the glomerular filtrate, such as albumin, or with secretion into the lumen from tubule cells. Hyaline casts are a characteristic of CPN.

Granular Cast (Figure 18)

Granular casts consist of non-homogeneous particulate matter representing cell breakdown products and debris. The casts are usually eosinophilic, but may be chromophobic. Typically they occur as a secondary consequence of necrosis or increased single-cell death affecting proximal tubule segments, as in α_{2U} -g nephropathy (1, 29).

Crystal formation (Figure 19)

Crystals may be deposited within the lumens of tubules, usually in the cortex or outer medulla. Due to the urine concentrating effect in the distal nephron, poorly soluble chemicals may form precipitates in this segment of tubule (22). The shape of crystals is specific for the chemical, but most remain transparent after conventional staining procedures. Some crystal types are rendered more visible by polarized light due to birefringence, while others may only be detected as empty clefts because of solubilization during histological processing (20, 22). Crystal deposition can lead to a special inflammatory process termed *obstructive nephropathy*. In this event crystals may become engulfed by multinucleated phagocytic cells. A number of drugs (e.g. sulfonamides, quinolone antibiotics, acyclovir) and chemicals (e.g. ethylene glycol), or their metabolites, are known to cause intraluminal crystal deposits (1, 22, 56).

Mineralization (Figures 20-24)

Mineralization in the rat kidney represents mainly calcium salt deposition. Depending on the severity, mineral deposits may be macroscopically visible as white or yellow stippling on the external or sectioned surface. Microscopically in H&E sections, mineralization appears as dense purple-staining deposits which can be of granular, concretionary, or linear form. The distribution can be random or zonal. Mineralization occurs more often in female than in male rats because of the relationship with estrogen levels, but is found in both very young and old animals (2, 20, 21, 51). Spontaneous occurrence frequently depends on the calcium-phosphorus ratio in the diet. Due to enhanced availability of minerals, some semipurified diets can lead to mineral deposition in tubules at the junction of outer and inner stripes of the outer medulla, while diets with high calcium-phosphorus ratios or with additional magnesium appear to have a protective effect (12, 22, 48). Several categories of mineralization can be distinguished depending on the location of the deposits, including tubular, intraluminal, basement membrane, interstitial and renal pelvic.

Tubular mineralization (Figure 20)

Tubular mineralization occurs as replacement of tubule epithelial cells by granular basophilic deposits, and is usually a consequence of tubule cell degeneration. Administration of phosphates, in particular, consistently results in mineralization of degenerating tubule cells (1). Although the characteristic mineralization occurring at the junction of outer and inner stripes of the outer medulla associated with certain diets in female rats commences as an intraluminal deposition (43), the lesions progress as cytoplasmic mineralization replacing the affected tubule cells. Ultrastructural observations on pathogenesis suggest that the early mineral deposits are formed from vesiculated microvilli and microvesicles shed from the proximal convoluted tubule, initiating intraluminal microlith formation (43). It is believed that the mineral deposits travel down the tubule lumen to become lodged because of increasing size at the junction of pars recta and thin descending limb of Henle. At this point, tubule epithelium in the immediate proximity of the luminal concretion undergoes degeneration and mineralization (43, 54).

Intraluminal mineralization (Figure 21)

The term intraluminal mineralization refers to mineral deposits that are confined to the tubule lumen, and these may be observed in the cortex, medulla or papilla. As indicated above the commonly observed mineralization occurring at the junction of outer and inner stripes of outer medulla in association with semi-purified or some high protein diets (48, 54) commences as intraluminal deposition. Intraluminal mineralization in tubules of the papilla has been referred to as *linear papillary mineralization*. Linear casts of basophilic granular material in the prebend segments of Henle's loop are characteristic of the chronic stage of chemically-induced α_{2U} -g nephropathy in male rats. In this condition, the mineral deposits have been identified as calcium hydroxyapatite (55), representing residues of granular casts formed in more active stages of injury. Linear papillary mineralization is often associated with hyperplasia of the epithelial lining of the papilla (1).

Basement membrane mineralization (Figure 22)

Mineralization of basement membrane appears as focal or, more usually, linear basophilic deposits preferentially involving the basement membranes of proximal convoluted tubules, blood vessels and/or glomerular tufts. This finding can be a typical indication of hypervitaminosis D, consumption of rodenticide containing cholecalciferol, or hyperphosphatemia due to chronic renal failure (1). As a consequence of the latter circumstance, basement membrane mineralization can be a feature of end-stage CPN.

Interstitial mineralization (Figure 23)

Interstitial mineralization occurs focally as small

microconcretions, most commonly in the interstitium of the cortex. A common form is the PAS-positive microconcretion displaying concentric lamellar patterns that originates from basement membranes of cortical tubules but becomes mineralized as ovoid or irregular deposits between tubules (7).

Renal pelvic mineralization (Figure 24)

The renal pelvis is commonly affected by mineralization, with mineral sediment being observed particularly in the calyceal fornices of the pelvis. The papillary tip may also show focal intratubular concretions or encrustations. The adjacent transitional epithelium can be ulcerated and/or hyperplastic, sometimes accompanied by focal fibrosis and granuloma formation in the surrounding parenchymal tissue. There is a strong association between substances that induce caecal enlargement, for example sucralose, and renal pelvis mineralization (38).

Renal Calculus

Renal calculi in the rat are well-formed mineral bodies usually composed of magnesium or calcium phosphate that occur in the renal pelvis. The process of calculus formation is termed *uroolithiasis*. Calculi can be attached to the urothelial surface or lie free in the renal pelvis lumen. The concretions consist of concentric laminations of densely basophilic material and they may be single or numerous, varying in size from small grit to large stones. Alterations that may be associated with renal calculi include transitional cell hyperplasia of the renal pelvis, vacuolar degeneration and formation of cyst-like spaces in the urothelium, hemorrhage, pyelonephritis and hydronephrosis. Renal calculi occur more often in aged than in young animals (8, 42).

Amyloidosis (Figure 25)

Although relatively common in Syrian hamsters and specific strains of mice, renal amyloidosis is extremely rare in rats (17). Microscopic findings include glomerular, peritubular and/or interstitial deposits of homogeneous, eosinophilic material, which stains positively with Congo red, and exhibits apple green birefringence with polarized light.

Glomerulosclerosis (Figure 26)

Glomerulosclerosis represents progressive replacement of glomerular matrix with amorphous hyaline material. Early changes include a thickened capillary basement membrane with an increased mesangial matrix. Segmental or global deposition of PAS-positive hyaline material progresses to dense, eosinophilic deposits with collapse and condensation of the capillary bed. Adhesions between glomerular visceral epithelium and parietal cells of Bowman's capsule may be present. Glomerulosclerosis in rats is a component of advanced CPN but it can be induced

experimentally by the administration of puromycin aminonucleoside.

CELL DEATH

Cells exhibiting irreversible structural alterations can be categorized into two distinct patterns, single cell death and necrosis. Cell death induced by xenobiotics can involve any cell population in the kidney, but most frequently affects the proximal tubule. Involvement of either proximal convolutions or pars recta may predominate depending on the mechanism of action of the toxicant. Toxic agents carried into the tubule bound to protein, such as lead, or associated with cellular protein overload, typically affect the proximal convolutions. Injury requiring metabolic activation of the chemical, can affect either the cortical tubules, as with chloroform, or involve mainly the pars recta, as with hexachlorobutadiene.

Single Cell Death (Figure 27)

Single cell death involves solitary, scattered cells within the epithelial lining of tubules or urothelium, or in the interstitium. When characterized by cell shrinkage, increased cytoplasmic eosinophilia, cytoplasmic blebbing at the luminal surface, and nuclear chromatin condensation and fragmentation, it is consistent with *apoptosis*. Single cell death usually does not elicit an inflammatory response but is accompanied by an increased mitotic rate. It is seen in proximal tubule epithelium as a consequence of exposure to xenobiotics that induce α_{2U} -g nephropathy, such as d-limonene and unleaded gasoline, and to subnecrogenic doses of lead salts or aminoglycoside antibiotics.

Tubule Necrosis (Figure 28)

Necrosis involves coagulative-type death of contiguous cells within tubules. The process may occur in multifocal or diffuse zonal distribution. The affected cells typically are swollen with decreased cytoplasmic staining, fragmented membranes, and karyorrhexis or nuclear pyknosis. Tubule necrosis may elicit an inflammatory response, which may be suppurative and focal, or more diffuse with scanty numbers of infiltrating mononuclear cells and proliferating fibroblasts. Tubule necrosis is typified by the zonal necrosis induced in the pars recta by inorganic mercuric salts or acetaminophen. Despite extensive injury, tubule necrosis may be completely reversible through the process of tubule regeneration.

Papillary Necrosis (Figures 29-30)

In papillary necrosis, the earliest changes consist of a loss of structural definition at the tip of the papilla, often progressing to involve the distal half or full depth of the papilla. At first the degenerative change involves the interstitial cells and blood vessels. Later the degenerative

change involves the tubule components. The necrotic papilla appears as a homogeneous, lightly eosinophilic matrix with ghost-like remnants of normal architecture, but without inflammation. The demarcation between necrotic papilla and the more proximal, surviving medullary tissue may show infiltration of inflammatory cells and/or mineralization. Sloughing of the necrotic papilla at this abscission zone leaves a broad, truncated surface, which undergoes re-epithelialization by transitional cells, often with hyperplasia (14). Secondary changes of tubule dilation and pyelonephritis may occur in the cortex. The severity of papillary necrosis can be graded according to the progression and extent of the lesion.

The rat is particularly susceptible to chemically-induced papillary necrosis. It is frequently seen after administration of non-steroidal anti-inflammatory drugs (NSAIDs), where the association presumably involves concentration of the xenobiotic and its metabolism in the renal papilla itself, predisposed by high interstitial solute concentration and a compromising of the regional blood flow. There may be a marked gender differentiation in predisposition to papillary necrosis induced by certain chemicals. For example, male rats develop a much more severe form than females in response to ethoxyquin (28).

INFLAMMATORY CHANGES

Glomerulonephritis

Renal disease produced by immunological mechanisms is not common in rats. Consequently, glomerular changes other than glomerulosclerosis or glomerular atrophy are rarely seen in this laboratory species. However, the potential exists for different types of glomerulonephritis, and the characteristics of each are described as supported by laboratory studies. Regardless of morphologic pattern, glomerulonephritis can be diffuse or focal/multifocal, and within each glomerulus the change can be global or segmental. Segmental versus global distribution may reflect the stage and severity of the disease process. Two micron paraffin sections, stained with periodic acid-Schiff (PAS) and/or appropriate silver techniques, are recommended for good resolution and definition of the morphologic pattern of the different types of glomerulonephritis.

Mesangioproliferative Glomerulonephritis

This is a diffuse glomerular lesion that is usually global, but sometimes segmental. It is characterized by hyperplasia and/or hypertrophy of mesangial cells, with an accompanying increase in mesangial matrix. The latter is PAS-positive but may or may not be positive for silver staining. An axial pattern of matrix expansion is usually apparent in the early lesion. In the advanced lesion, the accumulation of mesangial matrix is less axial and more generalized through the glomerulus. Glomerular capillary

loops are minimally affected. Secondary changes, such as thickening of Bowman's capsule and synechiae are variable. Mesangioproliferative glomerulonephritis has been induced in rats by administration of venom from the Habu snake (11).

Membranous Glomerulonephritis

This is a diffuse glomerular lesion characterized by normal cellularity along with a generalized and uniform thickening of glomerular basement membranes, particularly the capillary basement membrane. The basement membrane thickening represents deposition of aggregates of immune complexes along the capillary wall. There is no associated endothelial or epithelial proliferation although there may be a slight increase in mesangial matrix. Silver stains highlight the formation of linear densities and "spikes" along the glomerular basement membranes. Typical "wire loop" lesions may be prominent in either global or segmental distribution. Secondary changes are usually minimal. Membranous glomerulonephritis has been induced by chronic administration of low doses of mercuric chloride (32).

Crescentic Glomerulonephritis

This is usually a segmental, or at times circumferential, lesion characterized by the proliferation of parietal and/or visceral glomerular epithelial cells to form peripheral mats or crescents. Underlying proliferative tuft lesions commonly accompany the primary change. Adhesions between the glomerular tufts and the crescents are prominent. Bowman's space is reduced and intraglomerular matrix accumulation is variable. Crescentic glomerulonephritis has been induced in a rat model using anti-glomerular basement membrane serum (35).

Pyelonephritis (Figures 31-32)

Pyelonephritis is an acute inflammation of renal pelvis and parenchyma caused invariably by an ascending infection with bacteria. The term *pyelitis* may be used if the inflammatory process is restricted to the renal pelvis. Ascending infection is easily produced in rats because they have spontaneous vesicoureteral reflux. Thus, pyelonephritis may be the result of acute bladder inflammation, which ascends via the ureters to the renal pelvis. Inflammatory changes then ascend from the renal pelvis in a radial pattern to involve the cortex. Macroscopically in severe cases, the renal pelvis may be dilated with suppurative exudate and the tip of the papilla ulcerated and necrotic. The adjacent medulla is hyperemic, and whitish or yellow radial streaks may be visible in the cortex. Microscopically, ulceration and necrosis of the papilla is present and marked to severe infiltration of the medullary and cortical parenchyma by neutrophils is characteristic, particularly within cortical tubule lumens (13). The tubule epithelium in contact with intratubular plugs of neutro-

phils becomes basophilic and may show a pseudocomplex type of hyperplasia that is reversible (28). Acute pyelonephritis usually progresses over time to chronic inflammation with infiltration of mononuclear cells, including plasma cells and monocytes. Pyelonephritis can increase in severity and incidence with increasing age, but the spontaneous acute form is rare in the Fischer F344 rat (42). In addition to reflux of infectious organisms from the urinary bladder, causative factors of pyelonephritis include pelvic calculi and papillary necrosis.

Interstitial Nephritis (Figure 33)

Interstitial nephritis, which may be focal or generalized, consists of interstitial infiltrates between tubules of mononuclear inflammatory cells. The mononuclear infiltrates are dominated by lymphocytes, plasma cells and/or macrophages. Neutrophils are not a feature of interstitial nephritis, but there may be an accompanying increase in fibrocytic cells (fibrosis). In the focal form, there are solitary or scattered, discrete interstitial and/or perivascular aggregates of well-differentiated lymphocytes. The generalized form is characterized by patchy or diffuse distribution of mononuclear inflammatory cells. Focal interstitial nephritis must be differentiated from early lymphocytic leukemia, while the generalized form must be distinguished from pyelonephritis. Interstitial nephritis is also a secondary component of advancing CPN. Chronic administration of certain succinimide compounds is known to induce interstitial nephritis in rats (5).

Fibrosis (Figure 33)

Renal fibrosis represents a focal, patchy or generalized proliferation of resident fibrocytes within the interstitium, without conspicuous infiltration of inflammatory cells. If the fibrosis represents an acute reaction there is usually little collagen evident. In more chronic stages, collagen deposition in the interstitium may become a dominant feature for which the qualifying term *sclerotic* has been used.

Microabscess

Microabscesses are characterized by discrete microscopic aggregations of neutrophils, usually in the interstitial tissue. They may be either solitary or multifocal, and are often bilateral. Renal microabscesses are usually the result of systemically disseminated bacterial infection, occurring by direct embolism of pyogenic microorganisms in intertubular capillaries or glomerular loops.

VASCULAR CHANGES

Thrombosis (Figure 34)

Thrombosis is rare in rats unless secondary to valvular endocarditis, surgical techniques or pyelonephritis. Usually veins or venules are affected. The affected

vessel is occluded by a thrombus involving platelets and fibrin, which may become organized by myointimal cell proliferation, eventually with recanalization. Foci of dystrophic mineralization may also be present.

Infarction (Figures 35-36)

Infarction is a common sequela to thrombosis of the kidney and has acute and chronic stages. *Acute infarction* is typified by a wedge-shaped area of coagulative necrosis in the renal parenchyma. The extent of the acute lesion depends on the artery obstructed. Thus, arcuate arteries produce infarction extending from the medulla to the renal capsule; interlobular arteries produce the lesion only in the cortex (41). After an initial short period of congestion of the affected area, three distinct zones develop: central, peripheral, and marginal. The central zone shows coagulative necrosis. A rim of polymorphonuclear leukocytes separates this zone from the peripheral zone in which proximal tubules are lined by cells with eosinophilic, swollen cytoplasm and pyknotic nuclei. The outer marginal zone shows mainly congestion with normal glomeruli and perhaps a few necrotic proximal tubules. As the lesion progresses, the rim of neutrophils thickens, and fibroplasia commences at the periphery within 4 to 7 days. In the marginal zone tubular regeneration may be seen as early as 4 days.

In the stage of *chronic infarction*, the affected zone gradually collapses and is replaced by sclerotic connective tissue in which tubules are atrophied and glomeruli are crowded together. Mononuclear cell infiltration is generally present, and there may also be some dystrophic mineralization. Characteristically, in an old infarct the overlying renal capsule is depressed. These chronic forms of infarct have sometimes been termed nephritic scars (53).

Periarteritis

Periarteritis, which has also been called periarteritis nodosa and polyarteritis, affects muscular arteries, most frequently the pancreatic, mesenteric and spermatic arteries. The characteristic lesion is only occasionally seen in rat renal arteries (52). In the acute condition, segmental or global fibrinoid necrosis of the arterial wall occurs with disruption of the internal elastic lamina, along with a mainly neutrophilic inflammatory reaction with a few eosinophils. Chronic lesions are characterized by a perivascular, mixed chronic inflammatory cell infiltrate and the arterial wall is thickened with medial and adventitial fibrosis, leading to luminal narrowing or obliteration.

MISCELLANEOUS CHANGES

Tubule Basophilia (Figure 37)

The term tubule basophilia should be reserved for scattered tubule segments lined by basophilic epithelium without thickened basement membrane that are unrelated

to regeneration or evidence of antecedent tubule cell damage and/or necrosis. The specific entity implied by the use of this term should also exclude the basophilic tubules observed in CPN, particularly those characterizing the early stages, which are invariably associated with basement membrane thickening. It is likely that chemically-induced cytoplasmic basophilia in tubules is a reflection of an increase in ribosomal RNA.

Extramedullary Hematopoiesis (Figure 38)

In rat kidney, extramedullary hematopoiesis is mostly seen in the adipose tissue adjacent to the renal pelvis. Generally, the infiltration consists of differentiated erythroid or myeloid cells whereas erythroid stem cells contain small, darkly basophilic nuclei. As extramedullary hematopoiesis may be suggestive of leukemia, histological evaluation of other sites, such as spleen and liver, should assist differential diagnosis.

SPECIAL DISEASE PROCESSES

Chronic Progressive Nephropathy (CPN) (Figures 39-40)

CPN is a common spontaneous disease of aging rats. It occurs in both sexes, but is more prevalent and more severe in male rats. It is exacerbated by high protein diets. The earliest histologic change is a small and occasional focus of basophilic tubules with thickened basement membrane in the cortex, initially representing the adjacent convolutions of a single proximal tubule. This change is associated with an eosinophilic hyaline cast in the medullary segment of the same tubule. With progression, more tubules become affected and foci coalesce into areas of tubule alteration. Affected tubules display both degenerative and regenerative changes including simple hyperplasia. At the same time, dilated tubules filled with hyaline protein casts and lined by attenuated epithelium become particularly prominent in the medulla. With increasing severity, glomerular changes include condensation and collapse of capillary tufts, adhesions with Bowman's capsule, glomerular atrophy and sclerosis, progressing eventually to glomerular obsolescence. Interstitial changes include relatively mild, focal accumulations of mononuclear cells and varying degrees of fibrosis. In very advanced stages, little normal parenchyma remains in the cortex, cystic tubules are grossly conspicuous, and there may be uremic mineralization of basement membranes. The latter occurs because advanced disease is associated with systemic changes that include compensatory hyperplasia of the parathyroid glands with generalized mineralization, especially involving lungs and gastric mucosa. In safety evaluation studies CPN should be identified by this term and if appropriate, the severity should be graded. The lesion components of CPN, e.g. basophilic tubules with thickened basement membrane, hyaline casts, glomerulo-

sclerosis, interstitial nephritis etc., should not be individually diagnosed.

Alpha_{2U}-globulin Nephropathy

The hyaline droplet nephropathy induced exclusively in male rats by diverse substances, frequently light hydrocarbons, represents a unique sequence of renal changes (29, 37). Initially, these agents (e.g. d-limonene, decalin, unleaded gasoline) cause excessive accumulation of hyaline droplets in the P2 segment of proximal tubule, usually within several days of administration, and continuing with exposure. The accumulating protein can be identified as α_{2U} -g by immunohistochemistry. Sporadic single cell death, exfoliation of dead cells into the tubule lumen, and sporadic mitosis accompany hyaline droplet accumulation in the affected tubules. After several weeks, granular casts, representing aggregates of cell debris, form at the junction of the straight segment of proximal tubule and thin descending limb of Henle (i.e. the junction of inner and outer stripes of outer medulla). After several months of continuous exposure, intraluminal linear mineralization occurs in tubules of the renal papilla, usually accompanied by hyperplasia of the transitional cell lining of the papilla. The mineralization may be relatively severe by the end of a chronic study (29). When exposure is continued for a 2-year period, the nephropathy is typically associated with late developing atypical tubule cell hyperplasia and a low incidence of tubule cell tumors.

Obstructive Nephropathy (Figure 19)

Intraluminal crystal deposition causing tubule blockage can lead to an inflammatory process for which the term obstructive nephropathy is recommended. This is a degenerative/regenerative set of lesions involving a granulomatous type of inflammation, with tubule dilation proximal to the sites of obstruction. The granulomatous inflammation is characterized by interstitial infiltration of mononuclear inflammatory cells, and differentiation of monocytes into macrophages, epithelioid cells or multinucleated giant cells of the Langhans type. Neutrophils may also be present and there is usually a mild fibrosis. Phagocytosis of crystal deposits by the giant cells may be observed.

LOWER URINARY TRACT

Nonproliferative lesions of the ureters, bladder and urethra are grouped together under the subheadings of congenital, degenerative, inflammatory, vascular and miscellaneous changes.

CONGENITAL CHANGES

Ureteral Aplasia

Aplasia of the ureter ranges from focal under-

development to complete unilateral absence in conjunction with missing kidney and tubular portions of the genital tract. The condition appears to be rare, but can be induced in rat fetuses by administration of chlorcyclizine to pregnant females (36).

Hydroureter

Hydroureter is characterized by marked dilation of the ureter. It may be unilateral or bilateral. Congenitally the condition appears to be rare but it can occur as an acquired lesion due to blockage, sometimes resulting in secondary hydronephrosis. Hydroureter can be induced in fetuses by administration of thyroid stimulating hormone to pregnant females (36).

DEGENERATIVE CHANGES

Vacuolation

Vacuolation of the transitional epithelium is a non-specific lesion which has been described as a response to cell injury by bladder toxicants and carcinogens. It occurs in the cytoplasm of the transitional epithelial cells and usually is most prominent in the surface layer. Eosinophilic inclusions often lie within the vacuoles and may represent cytoplasmic degradative products. Vacuolation may also be artefactual, resulting from autolysis.

Mineralization

Mineralization occurs rarely in the lower urinary tract, but when it does, it usually involves the muscle wall of the urinary bladder. As with the kidney, mineralization in the lower urinary tract is represented by densely basophilic, amorphous/granular material.

Calculus (Figures 41-42)

Calculi, representing mineral concretions, can occur in the lumens of the ureters, urinary bladder or urethra of rats, but most commonly in the bladder. As in the kidney, the process resulting in calculus formation is termed *urolithiasis*. The most frequent site causing obstruction is the junction between ureter and bladder. Calculi can be round to oval in shape, with a smooth or faceted surface, consisting of concentric laminations of basophilic material. Calculi vary in size from fine sand, representing multiple particles, to large stones, which may be solitary. Very large stones can completely fill and distend the bladder lumen. In the rat, they are usually composed of magnesium ammonium phosphate. Calculi are often accompanied by chronic inflammation and sometimes they cause papillary and/or nodular urothelial hyperplasia, but are generally nonfatal. Calculi can occur spontaneously or may be induced by the administration of toxicants.

INFLAMMATORY CHANGES

Edema

Edema is characterized by the presence of eosinophilic proteinaceous fluid in the subepithelial connective tissue. It may occur independently or it may accompany acute inflammation. If fixative is inadvertently injected into the wall of the bladder during inflation at necropsy, the resulting artefactual change involving separation of epithelium from submucosa may be mistaken for edema.

Acute Inflammation (Figure 43)

Acute inflammation may be either suppurative or necrotizing, and may be accompanied by hemorrhage. In *suppurative inflammation*, neutrophils are present in the mucosa, subepithelial connective tissue and/or muscular coat of the lower urinary tract. *Necrotizing inflammation* is characterized by focal epithelial erosion involving only the mucosa, or deep ulceration extending through the entire urinary bladder wall. Inflammation of the lower urinary tract is commonly a response to epithelial irritation, which in turn can be induced by urinary crystals, calculi or toxic chemicals. Acute inflammation appears to be more common in female rats, possibly because the female urethra is shorter and straighter. In male rats, acute inflammation of the bladder is often secondary to acute prostatitis.

Chronic Inflammation (Figure 44)

Chronic inflammation is characterized by infiltration of the subepithelial connective tissue and muscle layer of the bladder wall with mononuclear cells. It may be accompanied by hyperplasia of the overlying urothelium. Chronic inflammation can be related to the presence of crystals or calculi in the bladder, or can be induced by the administration of urinary bladder toxicants.

VASCULAR CHANGES

Hemorrhage (Figure 43)

Hemorrhage is characterized by the presence of free red blood cells in the mucosa, subepithelial connective tissue, or the muscle layer of the lower urinary tract. It may occur as an entity by itself or in conjunction with acute inflammation. If hemorrhage occurs in the mucosa, the urine will often be discolored with a reddish tinge.

MISCELLANEOUS CHANGES

Proteinaceous Plug (Figure 45)

Proteinaceous plugs may be observed in the bladder and/or urethra of male rats, becoming more common with age. They are composed of eosinophilic (proteinaceous) material intermingled with exfoliated urothelial cells, and occasionally contain spermatozoa. Proteinaceous plugs

can be large, filling the bladder lumen, and their frequency in rats subject to necropsy may range from 5-30%. Plug formation is assumed to be the result of abnormal ejaculation and secretions from the male accessory sex glands (seminal vesicles, coagulating glands and prostate), which may occur during euthanasia. Therefore, these are not pathological lesions but represent mainly an agonal change. They are not precursors of bladder calculi and are considered to be incidental findings of no importance, the notation of which should be at the discretion of the pathologist.

Nematode Infection

Parasitic infestation of the urinary bladder by the nematode, *Trichosomoides crassicauda* (bladder threadworm), is common in wild rats, but rarely encountered in laboratory rats in recent years due to improved animal husbandry conditions (36). Histologically, sections of parasites may be free in the bladder lumen or attached to the mucosa. There is little or no inflammation other than the presence of globular leucocytes in the mucosa in some cases. Mucosal erosion and epithelial hyperplasia may also occur. Migrating larvae are found in tissues surrounding the kidney, ureters and urinary bladder (as well as in other organs), occurring in or around blood vessels, and causing focal hemorrhages as they pass through vessel walls. Female worms measure approximately 10 mm in length and 0.2 mm in diameter. Male worms are larval in character, measuring 1.5-3.5 mm in length. The majority of infected rats have less than three adult worm pairs in the urinary bladder (6). As the lung is the major pathway for migrating larva, this organ should also be checked for pulmonary congestion, hemorrhage and granuloma formation.

DISCUSSION

A topographical term that is commonly used inappropriately in the rat is *corticomedullary junction*. This term is most frequently used by pathologists to denote the boundary that is macroscopically visible upon section of the rat kidney, which is the junction between the outer and inner stripes of outer medulla. For the rat, corticomedullary junction should refer to the boundary between cortex and outer stripe of outer medulla. Another frequently used diagnosis that has not been recommended here is *tubule nephrosis*. The terms nephropathy and nephrosis are listed in medical dictionaries as being synonymous. However, in human nephrology, nephrosis more particularly connotes any degenerative kidney disease associated with non-inflammatory edema and albuminuria. In standardizing nomenclature for rodent kidney histopathology, the term nephropathy is recommended as being preferable to nephrosis because of its more general interpretation. However, it should be used in conjunction with an appropriate descriptor whenever possible, as for example, in hyaline

droplet nephropathy.

Attention to the methodologies for preparation and fixation of the kidneys and lower urinary tract is important for obtaining the maximum information from preserved tissues, and for the interpretation of any changes. In routine evaluation of kidneys, a longitudinal (sagittal) section through the midline including the tip of the papilla of the left kidney, and a transverse section across the middle of the right kidney (again through the papillary tip) followed by immersion fixation in 10% neutral buffered formalin (NBF), embedding in paraffin and sectioning to 4-5 micron thickness, is acceptable practice in safety evaluation studies. Enhanced microscopic evaluation and subtopographical location of lesions can be obtained by embedding formalin-fixed specimens in plastic, and cutting sections of 1-2 micron thickness. As artefactual change is inevitable with immersion fixation of kidney because of the instant collapse of tubule lumens and disruption of plasma membranes, a further level of microscopic definition can be obtained if kidneys are vascularly perfused in the functioning state (23). This can be done in the anesthetized rat via the abdominal aorta, first flushing out blood with anticoagulant (heparinized saline) followed by a combination fixative, usually including glutaraldehyde. The resulting fixation quality enhances light microscopic evaluation of cellular and interstitial changes, and is necessary for proper electronmicroscopic examination of the kidney. Because of the increasing gradient of solute concentration from cortex to papilla, the osmolality of the fixative solution must be adjusted appropriately for the kidney zone of interest. When the perfusate osmolality is not appropriate for cortex or outer stripe of outer medulla, perfusion fixation will produce cytoplasmic vacuolation in proximal tubule cells that is difficult to differentiate from hydroptic change at the light microscope level.

The ureters can be embedded on end, in which case microtomy provides a single cross section of each duct for microscopic examination. As this represents only a limited area for evaluation, a preferable method is to dissect out the ureters in their entirety, removing any serosal fat, coiling each ureter onto a small square of gelfoam, and immersing the specimen in NBF for fixation. After fixation, the square of gelfoam can be bisected and each half embedded on end. The gelfoam is sectioned in conjunction with the coiled ureter. This method provides multiple cross sections of each ureter for examination.

Because the epithelium of the urinary bladder readily undergoes autolysis, it should be fixed as rapidly as possible. If orientation of the urinary bladder is important in subsequent microscopic evaluation, the ventral or other surface can be identified with a dot of indelible ink (e.g. India ink) at the time of necropsy. The ink will remain on the bladder wall during processing and can be

visualized with the light microscope. For proper microscopic evaluation, the urinary bladder needs to be inflated with fixative, thus eliminating artefactual thickening of bladder mucosa and folds, and facilitating recognition of the presence or absence of hyperplasia. The urinary bladder of rodents can be inflated by one of two methods (18). The less effective of the two is injection of fixative directly into the lumen through the muscular wall with a small gauge (24-26 GA) needle. This sometimes results in the deposition of fixative into the bladder wall rather than into the lumen, resulting in microscopic distortion or loss of bladder epithelium, and making interpretation of urothelial status difficult. The preferred method is inflation through the urethra with a blunt needle. With both methods the bladder should be filled but not over-inflated. If the urinary bladder is already distended with urine, the contents can be first withdrawn using either method, and the bladder refilled with fixative. In male rats, the urinary bladder will usually stay inflated if the coagulating glands and seminal vesicles are removed *in toto* along with the urinary bladder. In females, it may be necessary to place a ligature around the neck of the bladder to prevent leakage. After inflation, the distended bladder should be immersed in fixative. For light microscopy, 10% NBF is an adequate fixative for inflation and immersion of the bladder, but for electron microscopy glutaraldehyde is recommended. Other fixatives, such as ethanol or Bouin's solution, may be preferable for specific immunohistochemical or *in situ* hybridization studies.

For most routine studies where histopathologic lesions of the urinary bladder are not anticipated, the inflated urinary bladder can be divided sagittally into two halves upon removal from the fixative, each being embedded with luminal side down into one paraffin block. A single section of the block will then provide two complete sagittal sections of the urinary bladder and urethra. If the urinary bladder is a known target organ, or if lesions are suspected at necropsy, the mucosa of the fixed bladder halves should be carefully examined with a dissecting microscope. Subsequent trimming of the tissue can then include any suspicious areas for sectioning. A second method of trimming that provides more mucosal surface for microscopic examination involves further division of each bladder half into 3 to 5 strips, which are embedded on end in a paraffin block (49). Since lesions in the urinary bladder mucosa may be extremely small and multifocal, serial sectioning through the whole bladder may sometimes be necessary. This procedure has been shown to significantly increase the identification of microscopic lesions.

NOMENCLATURE AND DIAGNOSTIC CRITERIA

KIDNEY

CONGENITAL CHANGES

Agenesis

1. Absence of one kidney

Hypoplasia

1. Usually unilateral
2. Diffuse or segmental
3. Reduced size, weight, and volume of kidney
4. Cortical fibrosis and cystic tubules
5. Hypoplastic changes in blood vessels

Polycystic Kidney

1. Usually bilateral
2. Diffuse symmetrical renal enlargement
3. Radially arranged dilated and cystic tubules in cortex and medulla
4. Few or no tubular casts
5. Atrophy of non-cystic cortical tubules
6. Interstitial fibrosis

Adrenal Rest

1. Small aggregation of adrenocortical cells
2. Attached to exterior of capsule or located subcapsularly
3. Surrounded by thin fibrous capsule

Hydronephrosis

1. Often unilateral, sometimes bilateral
2. Congenital or acquired
3. Varies from mild to severe dilation of renal pelvis with progressive parenchymal change
4. In mildest case, pelvis dilation causes no parenchymal changes
5. In severest case, kidney consists of fluid-filled sac with rim of atrophic cortex
6. Microscopically, compression of parenchyma with tubule atrophy, decreased glomeruli, glomerulosclerosis
7. Hemosiderin pigment and calcium concretions may be present in papilla
8. Calculi may be found in enlarged calyces
9. In congenital form, usually no evidence of urinary tract obstruction
10. In acquired form, evidence of urinary tract obstruction e.g. chronic inflammation

DISTURBANCES OF CELL GROWTH/DIFFERENTIATION

Glomerular Atrophy

1. Focal or multifocal, segmental or global

2. Contraction and shrinkage of one or more capillary tufts
3. Bowman's space often enlarged

Tubule Atrophy

1. Focal or multifocal, segmental or zonal
2. Contraction and collapse of tubule structure with obliteration of lumen
3. Tubule basement membrane markedly thickened
4. Varying degree of peritubular fibrosis

Cell/Tubule Hypertrophy

1. Enlarged tubules lined by single layer of enlarged cells
2. No increase in cell number
3. Cell shape cuboidal to columnar
4. Cytoplasm usually brightly eosinophilic but may be pale to oncocyctic
5. Nucleus often prominent in apical cytoplasm

Tubule Regeneration

1. Process restores tubule epithelium to normal after lethal injury
2. Tubule cells basophilic
3. Mitotic activity increased
4. May be a transition of cell shape from flattened to cuboidal
5. Cell number or tubule size not usually increased unless injury persistent

Karyomegaly/Karyocytomegaly/Multinucleation

1. Cells with enlarged vesicular nuclei or multiple nucleoli, sometimes with increase in cell size
2. Nuclei usually hyperchromatic with multiple nucleoli, sometimes pseudoinclusions
3. Enlarged cells bulge into tubule lumen
4. Affected cells usually solitary, occasionally in groups

Bowman's Capsule Metaplasia/Hyperplasia

1. Replacement of flat parietal cells of Bowman's capsule by high cuboidal epithelium
2. Accompanied by increase in number of parietal epithelial cells i.e. hyperplasia

Squamous Cell Metaplasia

1. Focal, multifocal or diffuse
2. Replacement of renal pelvis transitional epithelium by squamous cells
3. Nuclei usually oval or flattened in shape
4. Cellular axes parallel to basement membrane
5. Surface may be highly keratinized, non-keratinized or contain only keratohyaline granules
6. May be desquamation of cornified material
7. Sometimes nuclear pleomorphism and cellular atypia
8. Occasionally associated with hyperplasia of squamous cells

Osseous Metaplasia

1. Usually focal
2. Usually in cortex
3. Osseous tissue in interstitium displacing parenchyma
4. Irregular boundary with adjacent renal tissue
5. Unrelated to mineralization

DEGENERATIVE CHANGES

Vacuolation

1. Intracellular change, usually in epithelial cells
2. Discrete clear or translucent spaces of variable size in cytoplasm
3. Represents hydropic change or lipidosis

Hyaline Droplets

1. Eosinophilic, homogeneous cytoplasmic bodies
2. Variable in size and can replace and distort cytoplasm
3. Typically most prominent in proximal convoluted tubules sometimes extending into pars recta
4. Represent protein accumulation in phagolysosomes e.g. α_{2U} -g, lysozyme
5. When in pure form protein may crystallize producing polyangular configuration

Inclusion Bodies

1. Intracellular homogeneous profiles
2. Eosinophilic or basophilic

Intracytoplasmic

1. Restricted to cytoplasm
2. Represent myeloid bodies, giant mitochondria, or proliferating smooth ER

Intranuclear

1. Prominently located within nucleus

Pigmentation

1. Yellow to brown stippling or granules in cytoplasm
2. Represents intracellular deposits of lipofuscin, bilirubin or resorbed blood pigments (e.g. hemosiderin)
3. Requires special stains for specific differentiation of pigment type
4. Common in rats with advanced large granular cell lymphoma

Tubule Dilation

1. Focal, multifocal or diffuse

Simple Tubule Dilation

1. Mild to moderate expansion of tubule lumen
2. Tubule epithelium usually remains relatively normal
3. Radial or zonal distribution

Cystic Tubule

1. Markedly expanded tubule lumen
2. Lined with flattened single-cell layer
3. Variable lumen contents
4. Thin fibrous capsule occasionally

Tubular Cast

1. Uniform inclusions occupying a length or cross section of tubule lumen

Hyaline Cast

1. Homogeneous eosinophilic contents filling tubule lumen
2. Typically of protein composition

Granular Cast

1. Non-homogeneous contents of granular particulate matter
2. Eosinophilic or chromophobic
3. Typically consists of cell breakdown products and debris

Crystal Formation

1. Occurs in lumina of tubules
2. Usually located in cortex or outer medulla
3. Crystals sometimes rendered more visible by polarized light
4. Shape of crystals is chemical-specific
5. Crystals may be engulfed by multinucleated phagocytic cells
6. May lead to obstructive nephropathy

Mineralization

1. Typically consists of deposits of calcium salts
2. May be visible macroscopically as white or yellow stippling on outer surface or transection
3. Microscopically detectable as densely basophilic, slightly granular deposits
4. Can occur randomly or zonally, in cortex, medulla or papilla

Tubular mineralization

1. Granular basophilic deposits replacing the tubule cell
2. Usually results from tubule cell degeneration

Intraluminal mineralization

1. Basophilic granular deposits lying within tubule lumen
2. Can occur in cortex, medulla or papilla
3. Degenerative changes may affect tubule epithelium adjacent to deposits
4. In papilla, occurs as linear streaks of basophilic granular material, usually in prebend segment of Henle's loop (linear papillary mineralization)

Basement membrane mineralization

1. Focal or linear basophilic deposition in basement membranes

2. Preferentially involves proximal convoluted tubules, vessels and/or glomerular tufts

Interstitial mineralization

1. Rounded microconcretions in interstitium
2. Usually in cortex

Renal pelvic mineralization

1. Mineral deposits in the renal pelvis and/or edge of renal papilla, particularly in calyceal fornices
2. Adjacent transitional epithelium may show ulceration and hyperplasia
3. Sometimes accompanied by focal fibrosis and granuloma formation in adjacent parenchyma

Renal Calculus

1. Solitary or numerous
2. Mineral concretions attached to urothelium or lying free in lumen of renal pelvis
3. Consists of concentric laminations of densely basophilic material
4. Size varies from small grit to large stones
5. May be accompanied by chronic inflammation, ulceration and/or urothelial hyperplasia

Amyloidosis

1. Glomerular, peritubular and/or interstitial deposits of homogeneous eosinophilic material
2. Congo red positive, apple-green birefringence
3. Very rare in rats

Glomerulosclerosis

1. Progressive replacement of glomerular structure with amorphous hyaline material
2. Early changes segmental, including thickened capillary basement membranes and increased mesangial matrix
3. Advanced changes global, including glomerular contraction and collapse
4. May be adhesions between visceral and parietal epithelium

CELL DEATH***Single Cell Death***

1. Involves individual cells, may be solitary or scattered
2. Increased cytoplasmic eosinophilia
3. Nuclear changes variable, may include peripheral condensation of chromatin and fragmentation
4. Absence of inflammatory response

Tubule Necrosis

1. Multifocal or diffuse zonal distribution
2. Coagulative-type death of contiguous cells in tubules, frequently involving adjacent tubule cross sections
3. Cells swollen with decreased cytoplasmic staining

4. Nuclear changes include pyknosis, fragmentation, or karyorrhexis
5. Presence or absence of inflammatory response

Papillary Necrosis

1. Earliest stage is loss of structural definition at papilla tip involving mainly interstitial cells
2. Progresses to loss of microvasculature, loops of Henle, collecting ducts with replacement by homogeneous eosinophilic matrix
3. Severest form involves confluent necrosis extending from tip through the full papilla
4. Mineralization and/or inflammation may occur in transverse band between necrotic and viable tissue (abscission zone)
5. Sloughing of necrotic papilla may occur at abscission zone, followed by re-epithelization of surface
6. Re-epithelialized surface may become hyperplastic
7. Secondary changes include pyelonephritis and tubule dilation
8. Severity can be graded according to extent of papilla involvement

INFLAMMATORY CHANGES

Glomerulonephritis

Mesangioproliferative Glomerulonephritis

1. Hyperplasia/hypertrophy of mesangial cells
2. Increase in mesangial matrix (PAS-positive)
3. Prominent axial pattern in early lesion
4. Essentially normal capillary basement membranes

Membranous Glomerulonephritis

1. Generalized uniform thickening of capillary basement membranes
2. Linear densities and “spikes” along glomerular basement membrane (argyrophilic)
3. Densities represent immune complex deposits
4. May be segmental or global “wire loop” lesions
5. Normal glomerular cellularity

Crescentic Glomerulonephritis

1. Peripheral mats or crescents of proliferating parietal and/or visceral epithelial cells
2. Adhesions of glomerular tufts to crescents
3. Proliferative tuftal lesions may occur
4. Reduction of Bowman’s space

Pyelonephritis

1. Acute inflammation of renal pelvis and parenchyma involving neutrophils
2. Inflammatory changes usually ascend from renal pelvis in radial pattern to involve cortex
3. Marked to severe infiltration of renal tissue by neutrophils, particularly into cortical tubule lumens
4. Tubule epithelium adjacent to intraluminal

- neutrophils basophilic and hyperplastic
5. Usually progresses to chronic inflammation with infiltration of lymphocytes, plasma cells and monocytes
6. Tip of papilla may be ulcerated and necrotic

Interstitial Nephritis

1. Focal (solitary, scattered, or perivascular) or generalized (patchy or diffuse)
2. Interstitial infiltrates of mononuclear inflammatory cells (lymphocytes, plasma cells, macrophages)
3. May be an increase in fibrocytes
4. Must be differentiated from lymphocytic leukemia, pyelonephritis, CPN

Fibrosis

1. Focal, patchy or diffuse increase in interstitial fibrocytes
2. Inflammatory cells not conspicuous
3. In chronic stages, interstitial collagen deposition may predominate over cellular component (sclerosis)

Microabscess

1. Solitary or multifocal
2. Discrete microscopic aggregations of neutrophils
3. Usually present in interstitial tissue associated with bacterial emboli

VASCULAR CHANGES

Thrombosis

1. Intravascular occlusion by a clot
2. Clot composed of platelets and fibrin
3. Can be recent, organized or recanalized

Infarction

Acute infarction

1. Consists of central, peripheral and marginal zones
2. Wedge-shaped area of coagulative necrosis in parenchyma centrally
3. Rim of neutrophil infiltration with tubule degeneration peripherally
4. Mainly congestion in marginal zone

Chronic infarction

1. Shrinkage of affected area
2. Progressive tubule atrophy, interstitial fibrosis, glomerular crowding
3. May be mononuclear cell infiltration and mineralization
4. Depressed renal capsule on outer surface

Periarteritis

1. Occurs in muscular arteries
2. Acute stage characterized by fibrinoid necrosis (segmental/global)
3. Mixed inflammatory cell infiltration
4. Chronic stage characterized by perivascular chronic

- inflammatory cell infiltration
- 5. Medial and adventitial fibrosis
- 6. Leads to narrowing or obliteration of vessel lumen
- 7. Sometimes thrombosis

MISCELLANEOUS CHANGES

Tubule Basophilia

1. Usually multifocal
2. Tubule basophilia without basement membrane thickening
3. Unrelated to antecedent tubule necrosis and regeneration
4. Distinct from basophilic tubules in CPN

Extramedullary hematopoiesis

1. Located in fat tissue adjacent to renal pelvis
2. Consists of differentiated erythroid and myeloid cells
3. To be distinguished from leukemia

SPECIAL DISEASE PROCESSES

Chronic Progressive Nephropathy (CPN)

1. Common spontaneous degenerative disease of aging rats
2. More common and more severe in male rats
3. Earliest histological lesion consists of occasional focus of basophilic tubules with thickened basement membranes and a hyaline cast further down the nephron
4. Tubule changes represent degeneration/regeneration/compensatory hyperplasia
5. With progression, segmental tubule atrophy, collapse and/or tubule dilation in cortex with prominent hyaline casts in medulla
6. Progressive glomerular sclerosis and atrophy
7. Interstitial changes include mononuclear cell foci and fibrosis
8. Advanced disease associated with parathyroid gland hyperplasia and widespread mineralization

Alpha_{2U}-globulin Nephropathy

1. Occurs exclusively in mature male rats
2. Epithelial cells of P2 segment show excessive hyaline droplet accumulation
3. Hyaline droplets stain positively for α_{2U} -g
4. Associated with single cell death, single cell exfoliation, and mitosis in proximal tubules
5. Granular casts in tubules at junction of outer and inner stripes after several weeks
6. Linear mineralization in tubules (descending Henle limb) of papilla after several months
7. Papillary mineralization often associated with urothelial hyperplasia
8. Typically associated with focal atypical tubule hyperplasia and low incidence of renal tubule

tumors after chronic exposure

Obstructive Nephropathy

1. Inflammatory process resulting from tubule blockage by crystals
2. Characterized by granulomatous inflammation and tubule dilation
3. Infiltrating monocytes may differentiate into epithelioid cells and Langhans giant cells
4. Giant cells may phagocytize crystal deposits

LOWER URINARY TRACT

CONGENITAL CHANGES

Ureteral Aplasia

1. Focal or complete
2. Focal, ureter focally undeveloped
3. Complete, unilateral absence of ureter as well as kidney and tubular portions of genital tract

Hydroureter

1. Unilateral or bilateral
2. Spontaneous or acquired
3. Marked dilation of ureter

DEGENERATIVE CHANGES

Vacuolation

1. Vacuoles in cytoplasm of transitional epithelial cells
2. May have eosinophilic contents
3. Most prominent in superficial epithelial layer

Mineralization

1. Densely basophilic amorphous/granular material
2. Usually located in muscle wall of bladder

Calculus

1. Solitary or multiple
2. Mineral concretion attached to mucosa or free in lumen of ureter, urinary bladder or urethra
3. Consists of concentric laminations of basophilic material
4. Size varies from fine sand to large stones
5. Stones may completely fill and distend bladder lumen
6. Obstruction, hydroureter, inflammation, hyperplasia may occur secondarily

INFLAMMATORY CHANGES

Edema

1. Presence of eosinophilic proteinaceous fluid in subepithelial connective tissue
2. May be accompanied by acute inflammation

Acute Inflammation**Suppurative inflammation**

1. Presence of neutrophils within lumen, mucosa, subepithelial connective tissue and/or muscle wall
2. May be associated with hemorrhage

Necrotizing inflammation

1. Focal mucosal erosion or deep ulceration through bladder wall
2. May be associated with hemorrhage

Chronic Inflammation

1. Infiltration of mononuclear inflammatory cells into subepithelial connective tissue
2. May be accompanied by urothelial hyperplasia

VASCULAR CHANGES**Hemorrhage**

1. Presence of free red blood cells in mucosa, subepithelial connective tissue and/or muscle wall
2. May occur alone or accompany acute inflammation
3. Urine tinged red if mucosa affected

MISCELLANEOUS CHANGES**Proteinaceous Plug**

1. Eosinophilic proteinaceous material intermingled with exfoliated urothelial cells in male rat bladder and/or urethra
2. Occasionally contains spermatozoa
3. May be large, filling bladder lumen
4. Represents agonal secretion from sex glands

Nematode Infection

1. Caused by *Trichosomoides crassicauda*
2. Sections of parasite either free in bladder or attached to mucosa
3. Globular leukocytes may be present in mucosa
4. Little or no inflammatory response present
5. Mucosal erosion and epithelial hyperplasia may be present

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Fig. 1 - Hypoplasia (H&E).

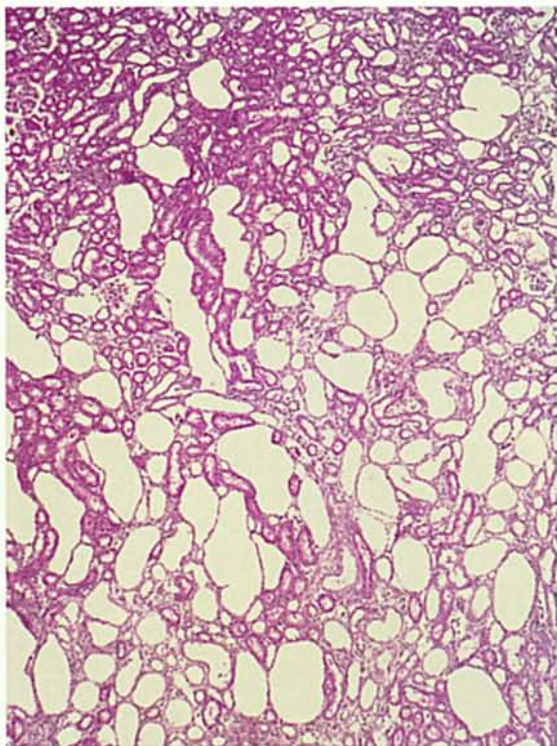


Fig. 2 - Polycystic kidney (H&E).

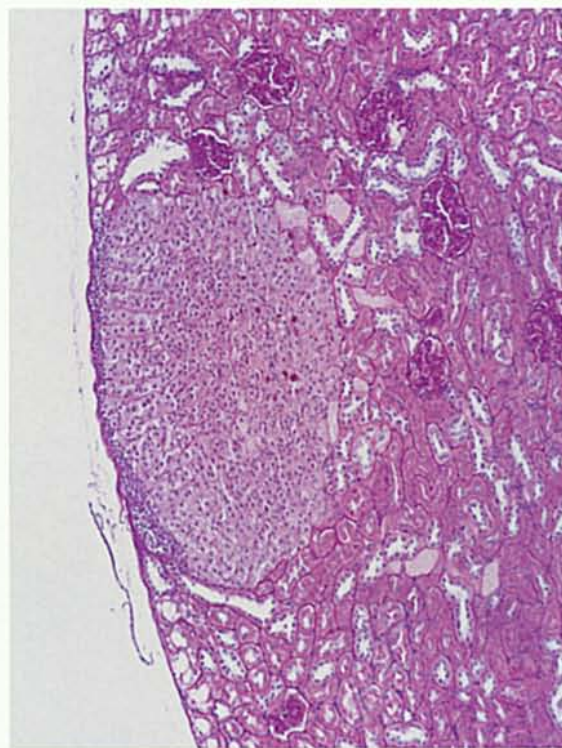


Fig. 3 - Adrenal rest (H&E).

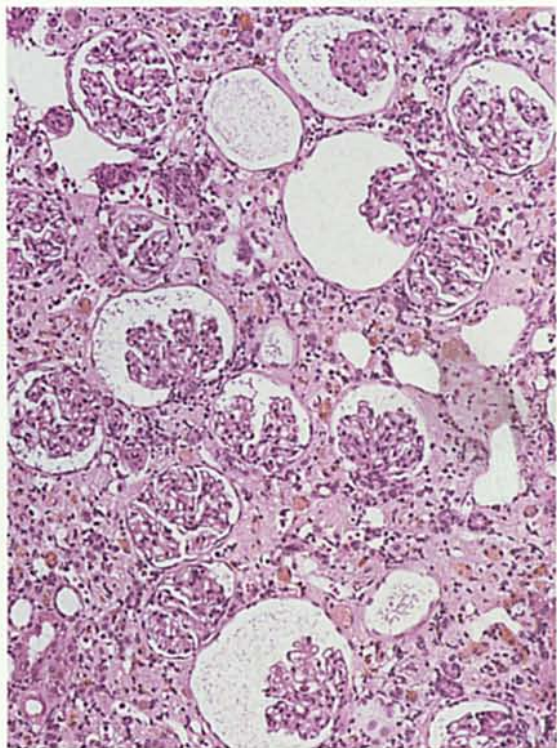


Fig. 4 - Glomerular atrophy (H&E).

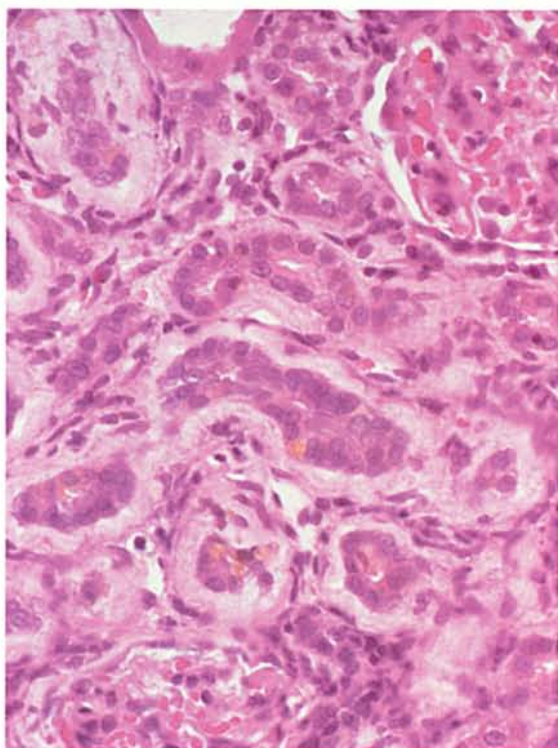


Fig. 5 - Tubule atrophy (H&E).

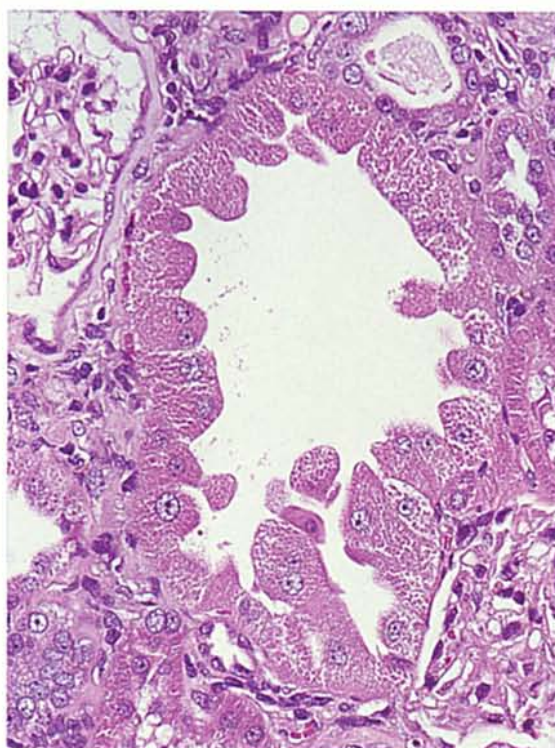


Fig. 6 - Tubule cell hypertrophy (H&E).

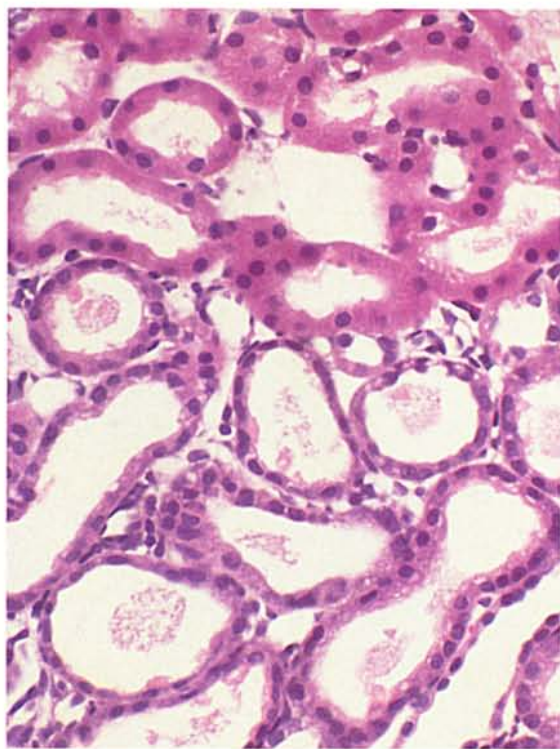


Fig. 7 - Tubule regeneration (H&E).

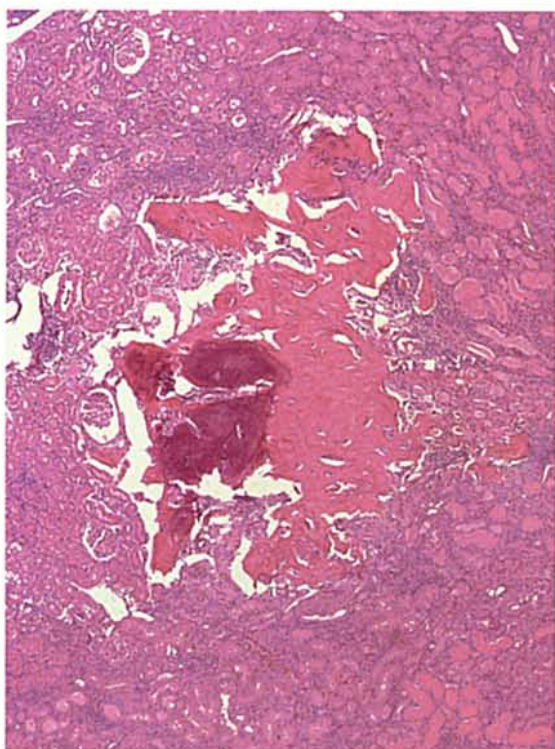


Fig. 8 - Osseous metaplasia (H&E).

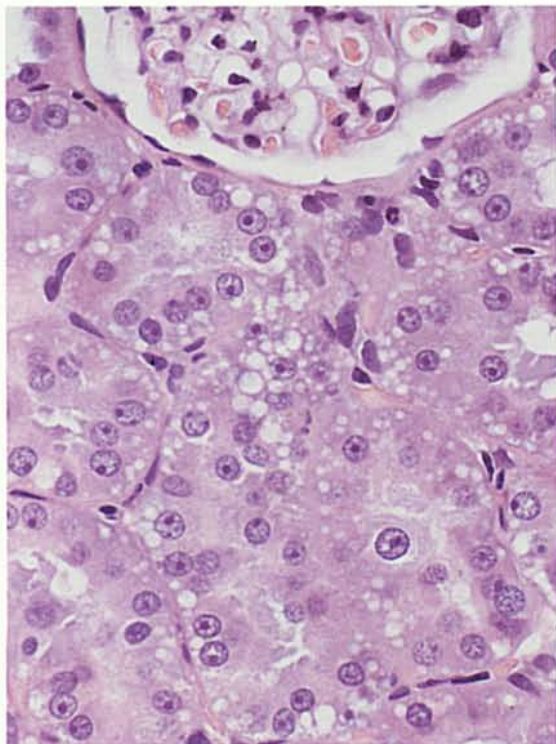


Fig. 9 - Vacuolation, proximal tubules (H&E).

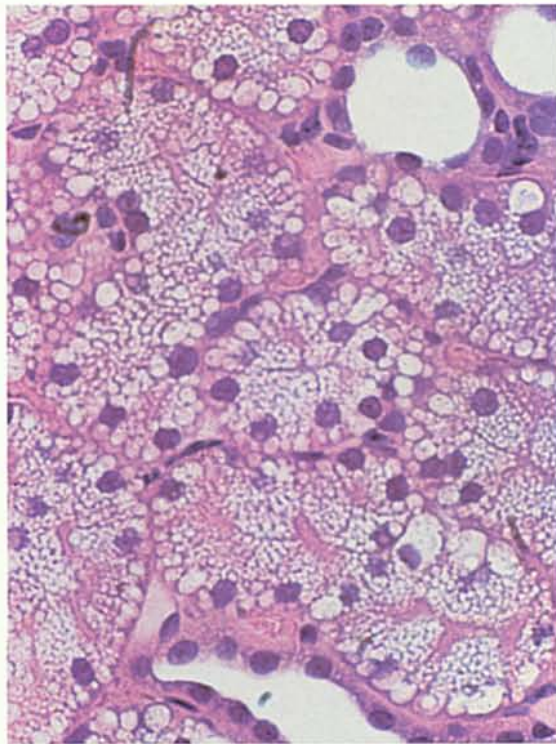


Fig. 10 - Vacuolation - hydropic change, proximal tubules (H&E).

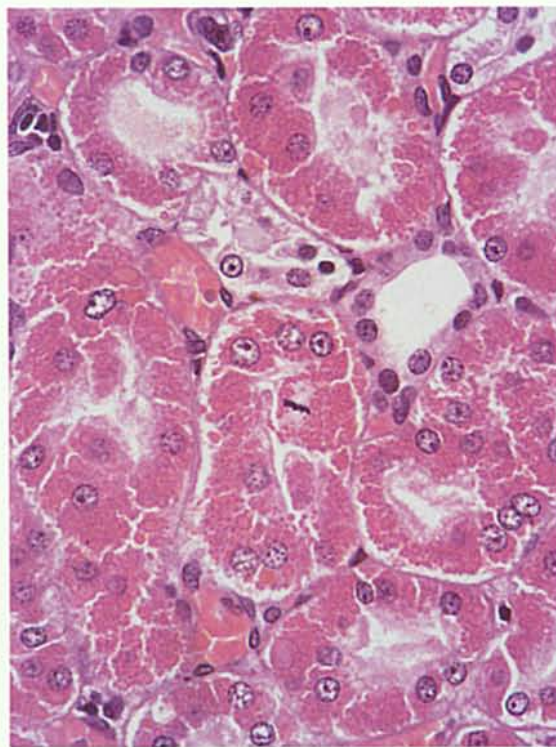


Fig. 11 - Hyaline droplets, proximal tubules (H&E).

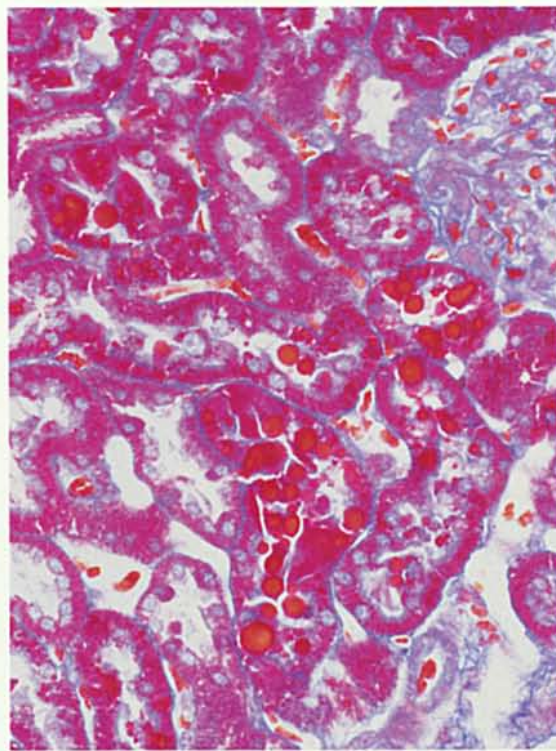


Fig. 12 - Hyaline droplets, proximal tubules (Mallory Heidenhain).

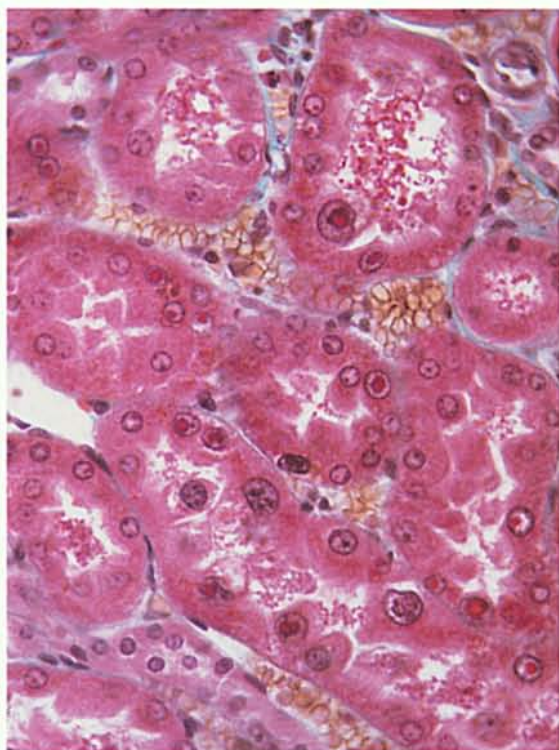


Fig. 13 - Intranuclear inclusions, proximal tubules (modified Trichrome).

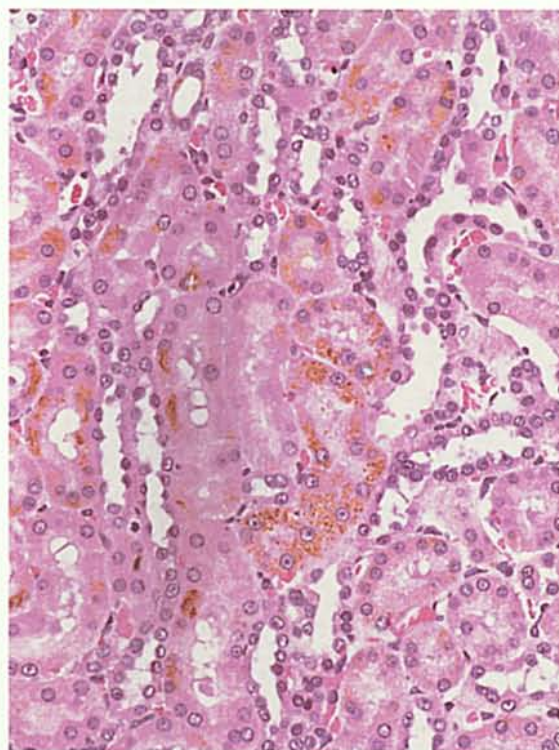


Fig. 14 - Pigmentation, proximal tubules (H&E).

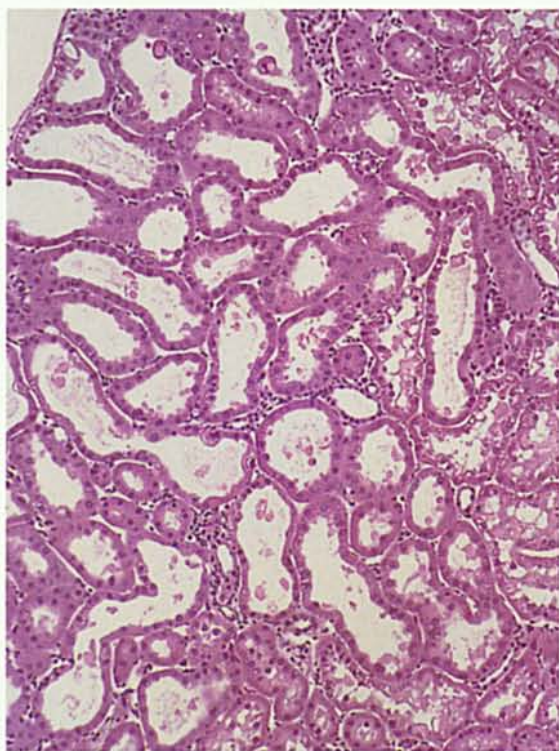


Fig. 15 - Simple tubule dilation (H&E).

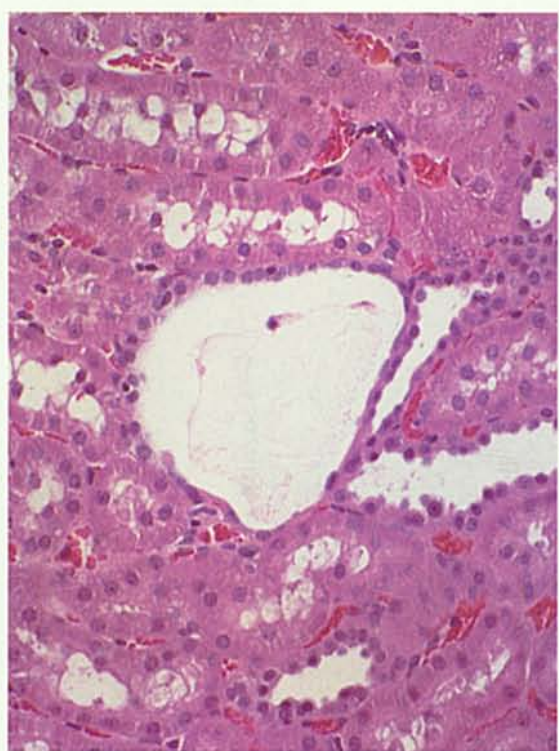


Fig. 16 - Cystic tubule (H&E).

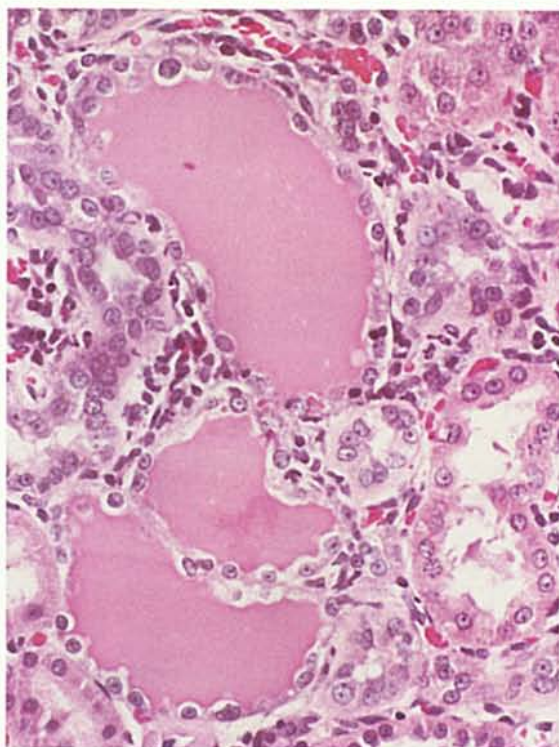


Fig. 17 - Hyaline cast (H&E).

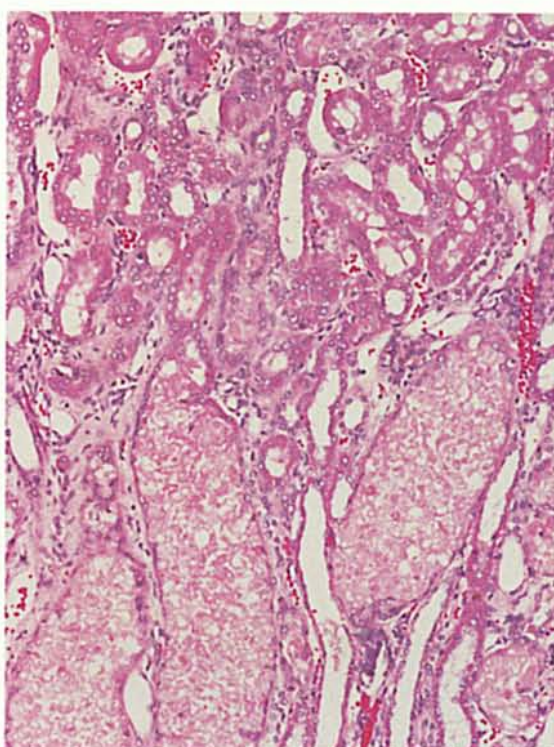


Fig. 18 - Granular cast (H&E).

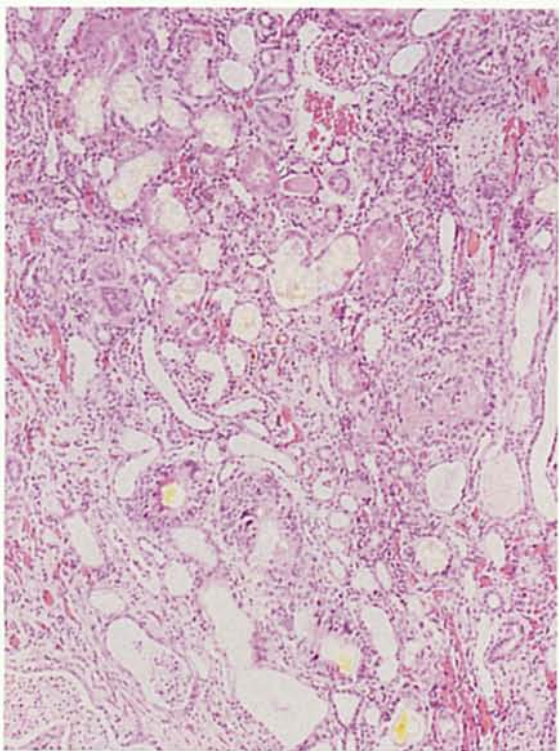


Fig. 19 - Crystal formation and obstructive nephropathy (H&E).

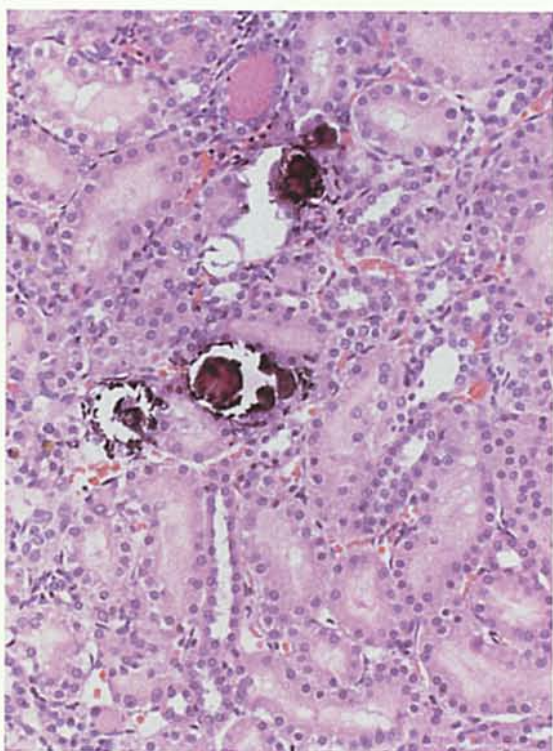


Fig. 20 - Mineralization, tubular (H&E).

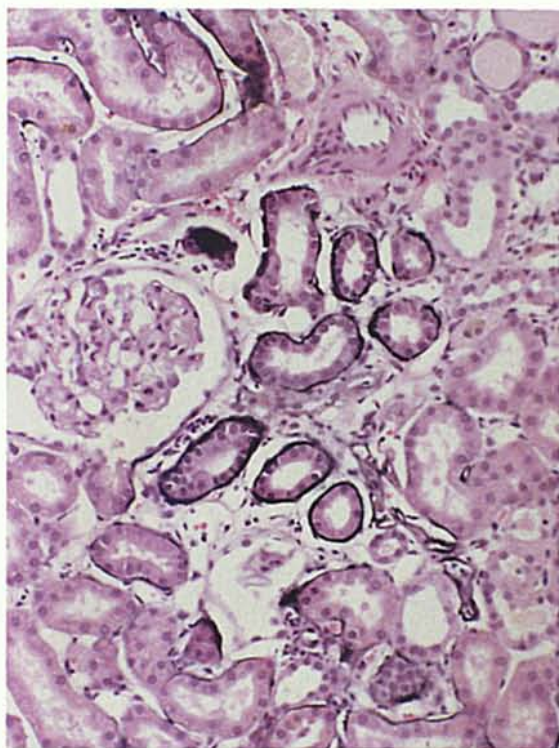


Fig. 21 - Mineralization, intraluminal (H&E).

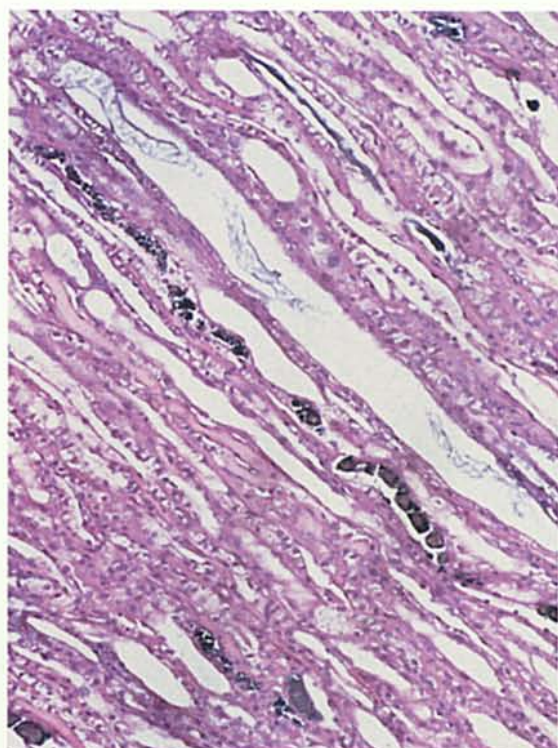


Fig. 22 - Mineralization, basement membrane (H&E).

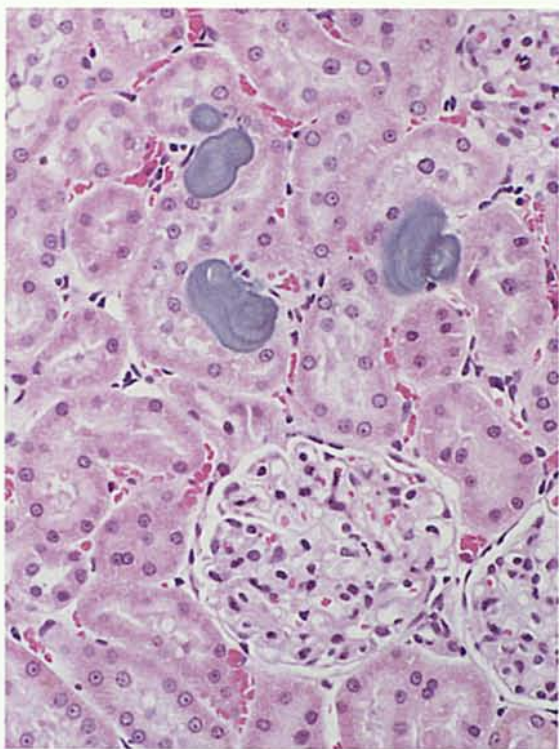


Fig. 23 - Mineralization, interstitial (H&E).



Fig. 24 - Mineralization, renal pelvis (H&E).

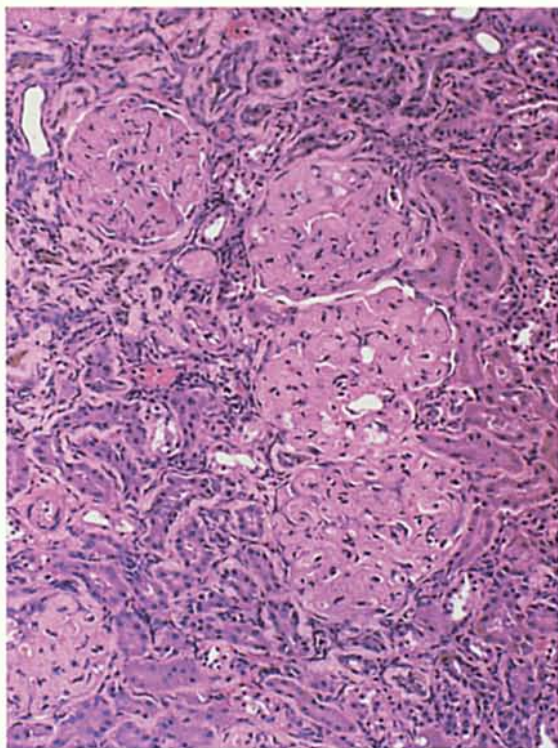


Fig. 25 - Amyloidosis, glomerular (H&E).

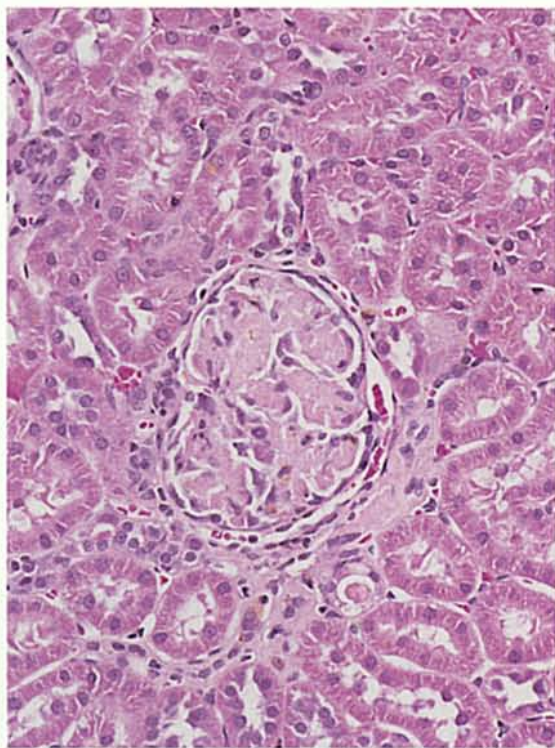


Fig. 26 - Glomerulosclerosis (H&E).

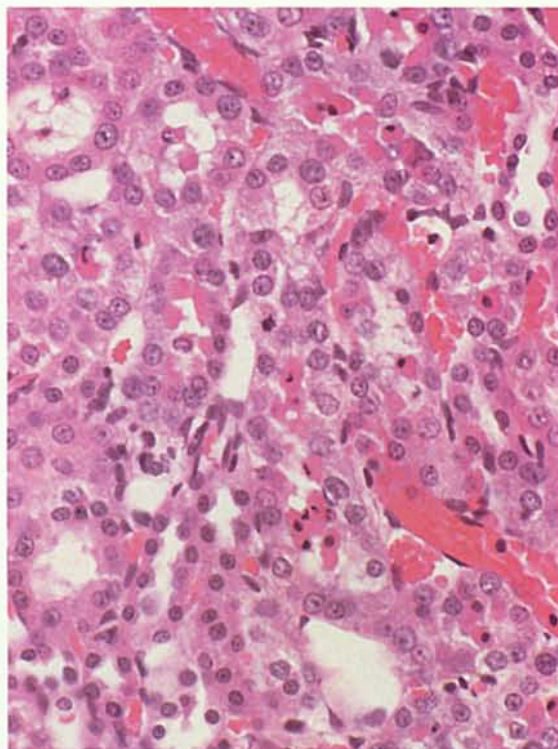


Fig. 27 - Single cell death, proximal tubule (H&E).

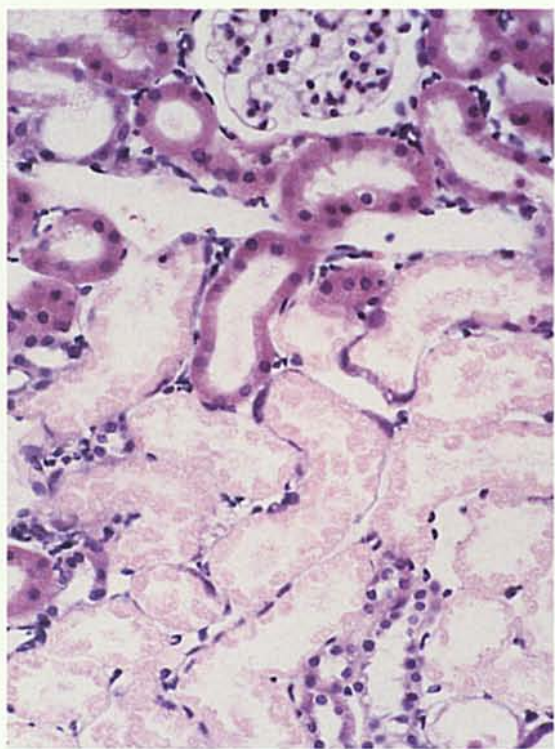


Fig. 28 - Tubule necrosis (H&E).

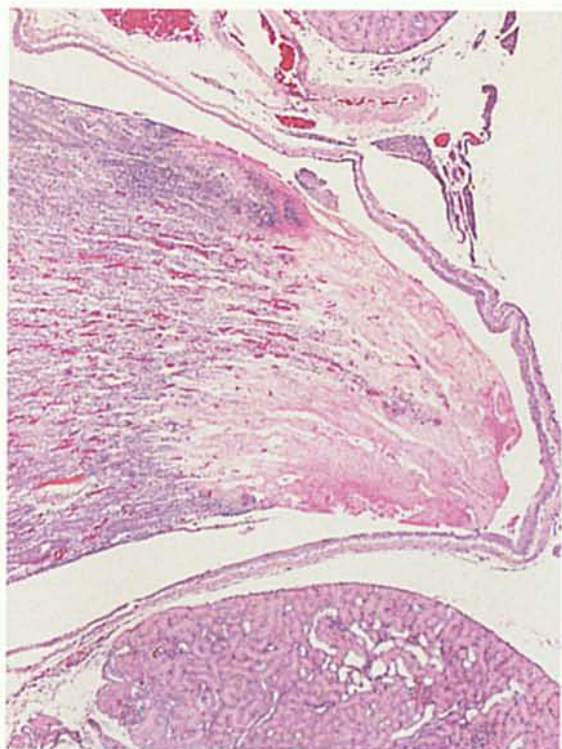


Fig. 29 - Papillary necrosis (H&E).

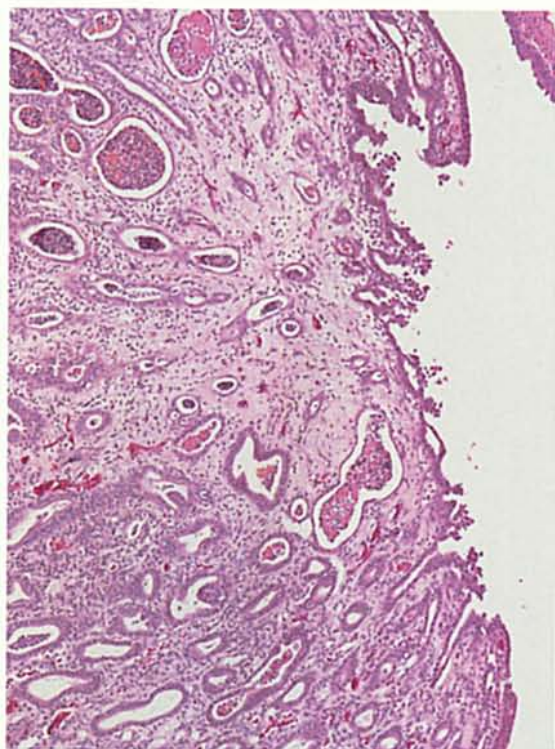


Fig. 30 - Papillary necrosis, resolved after sloughing of tip (H&E).

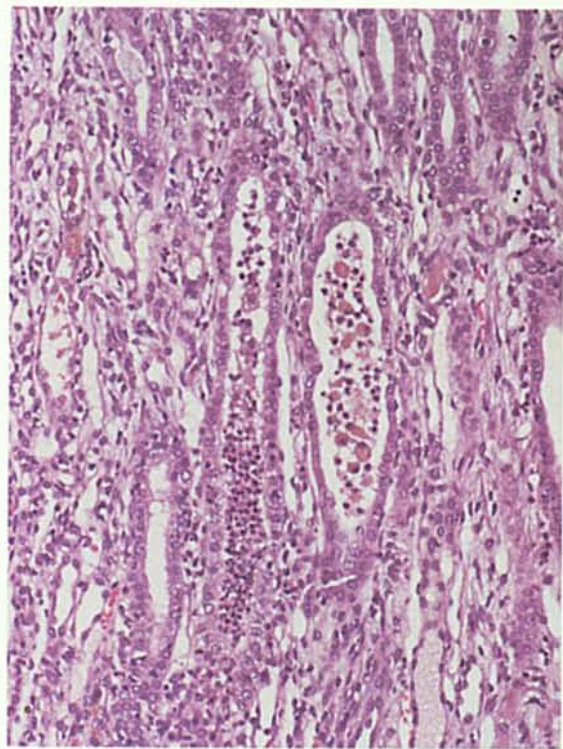


Fig. 31 - Pyelonephritis (H&E)

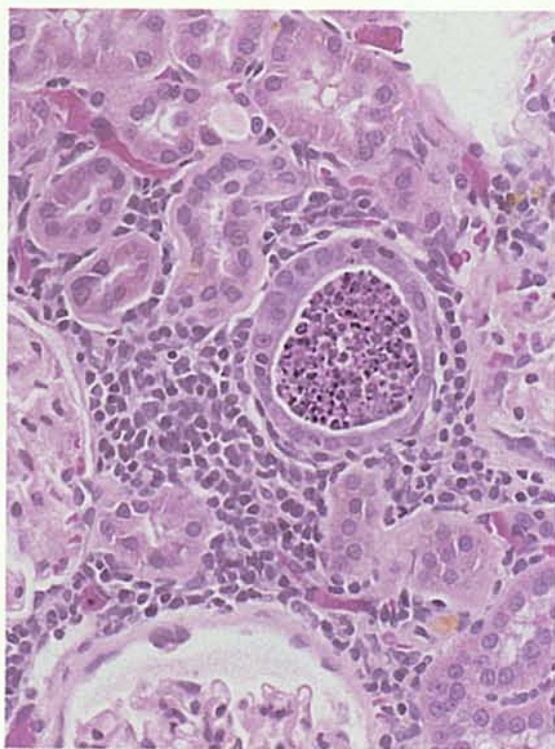


Fig. 32 - Pyelonephritis, with mononuclear interstitial inflammation (H&E).

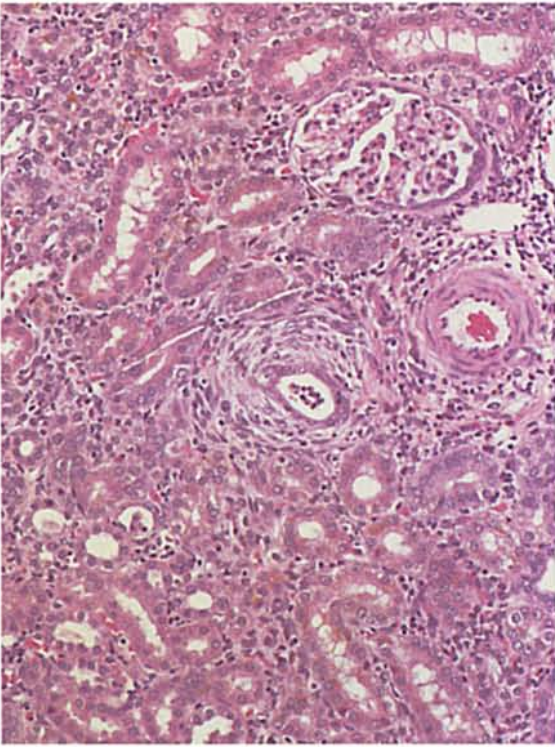


Fig. 33 - Fibrosis, peritubular, and interstitial nephritis (H&E).

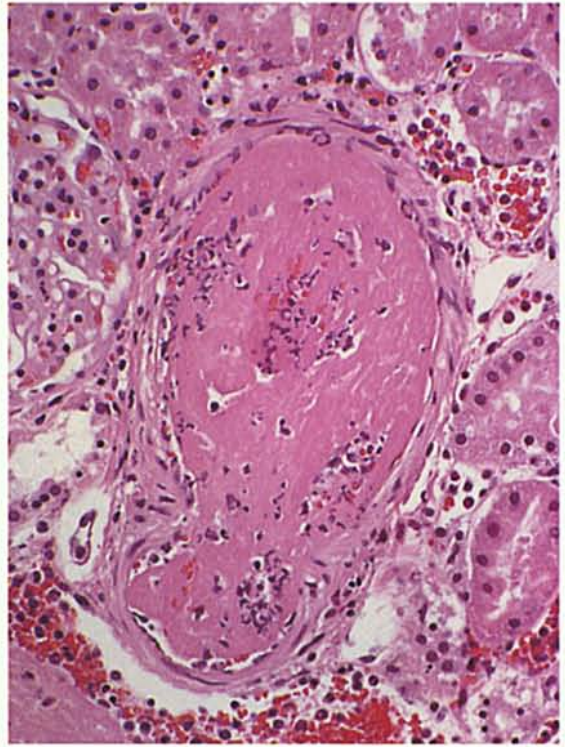


Fig. 34 - Thrombosis (H&E).

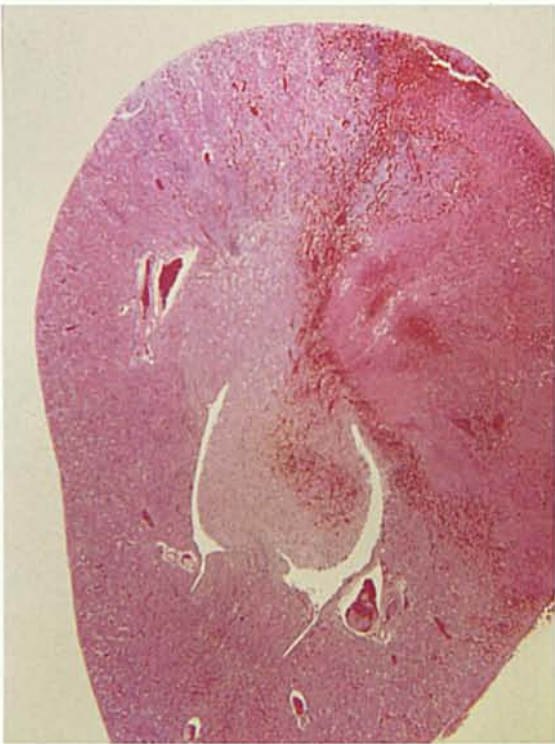


Fig. 35 - Infarction, acute.

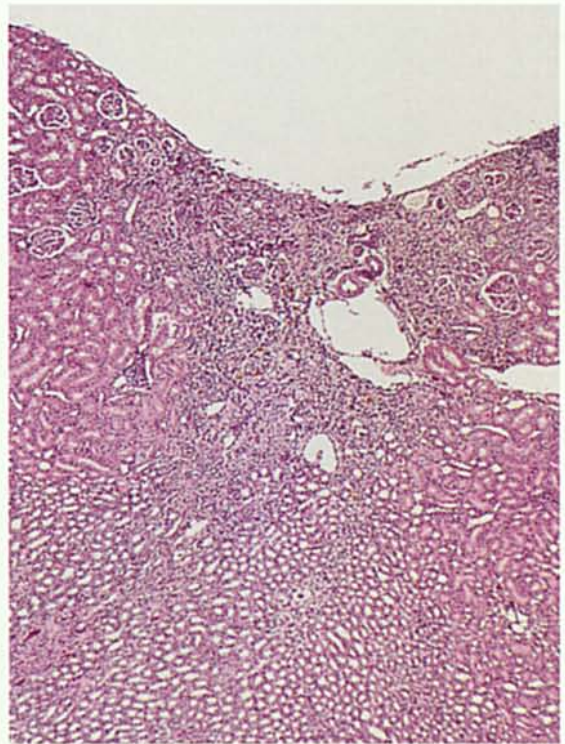


Fig. 36 - Infarction, chronic (H&E).

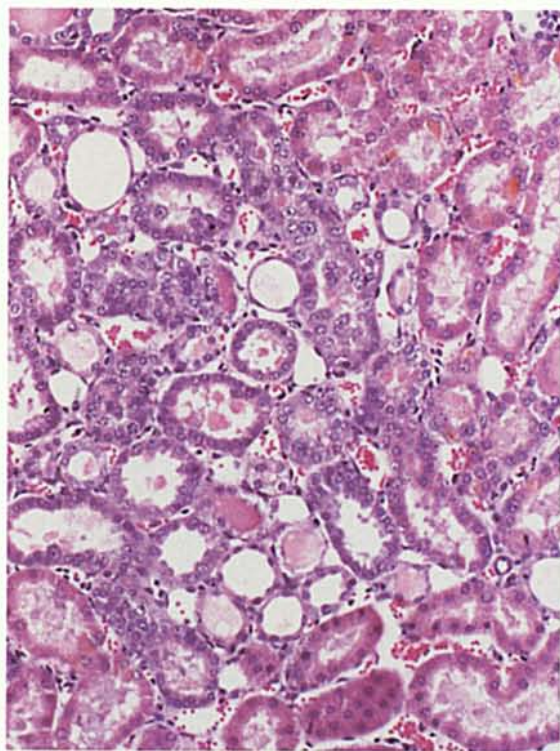


Fig. 37 - Tubule basophilia (H&E).

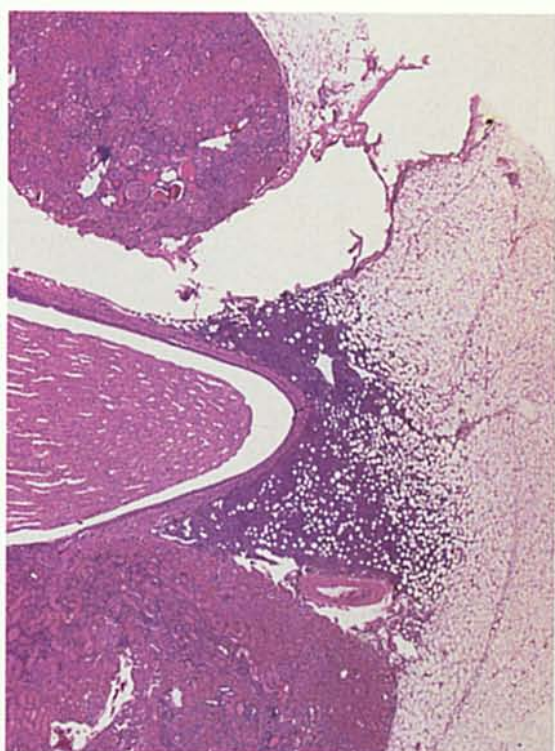


Fig. 38 - Extramedullary hematopoiesis (H&E).

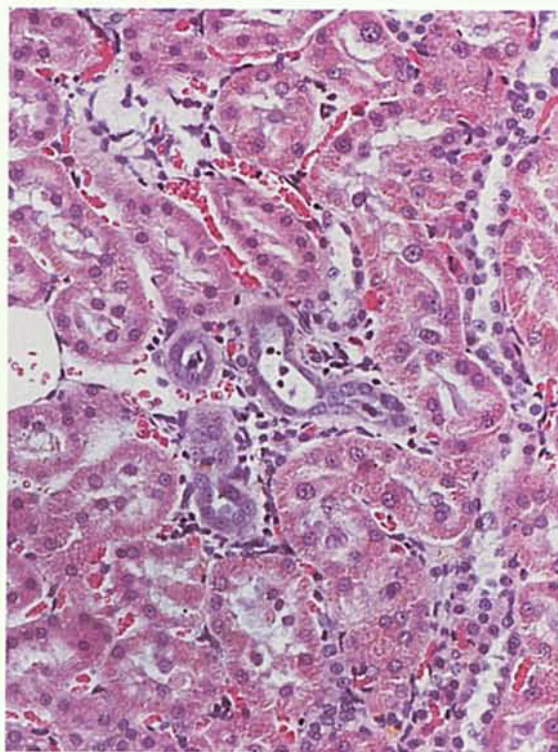


Fig. 39 - Chronic progressive nephropathy, early stage (H&E).

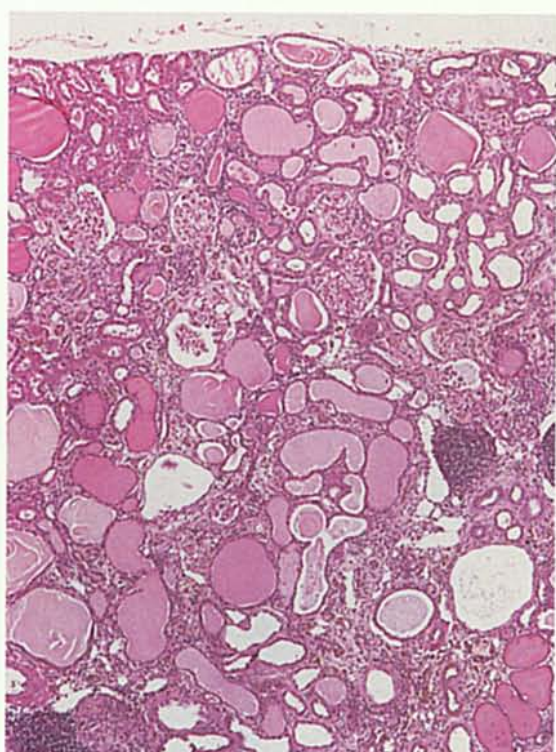


Fig. 40 - Chronic progressive nephropathy, advanced stage (H&E).

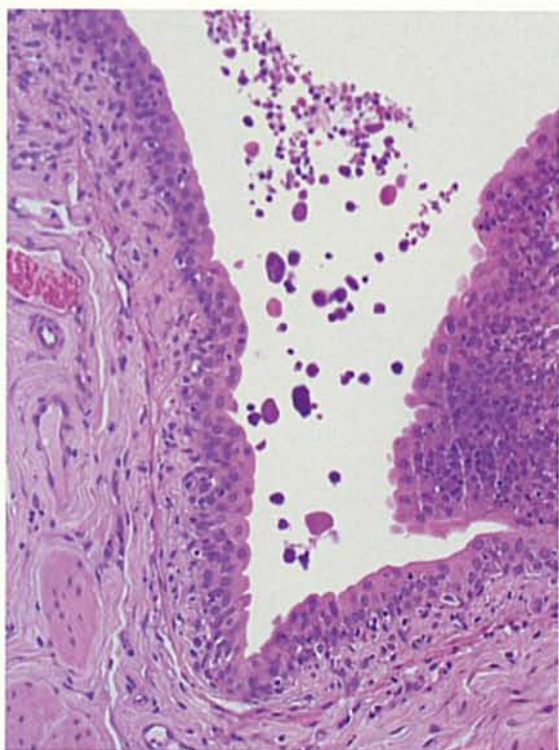


Fig. 41 - Calculi, bladder (H&E).

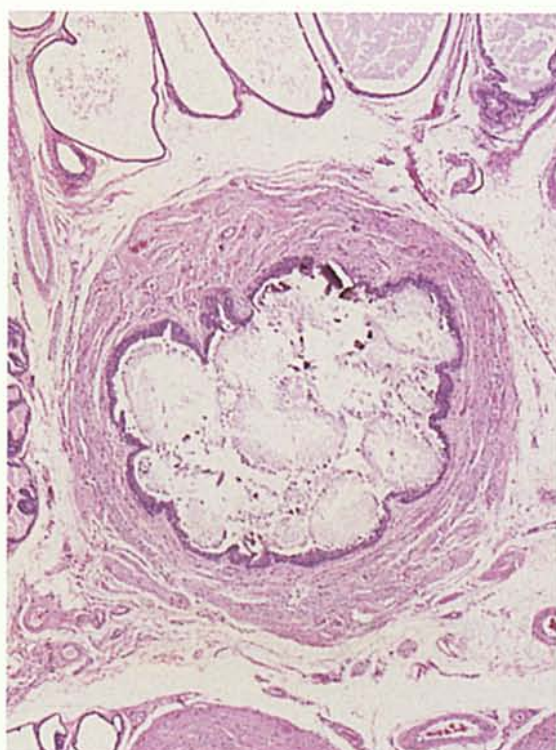


Fig. 42 - Calculus, urethra (H&E).

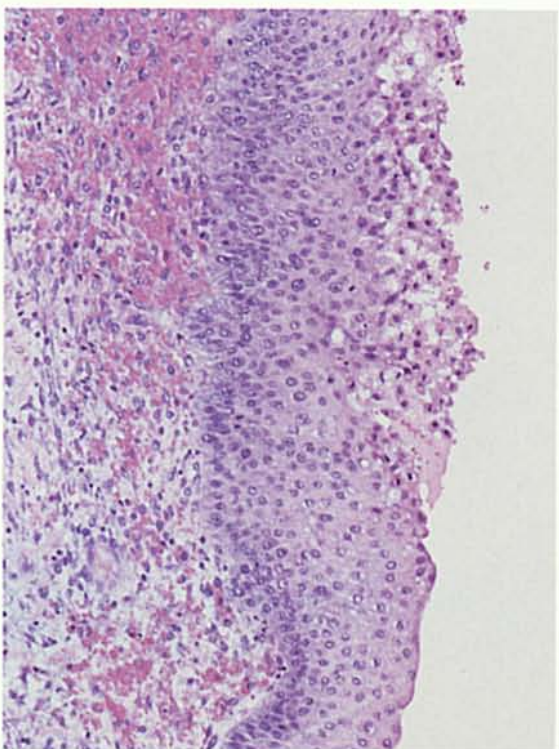


Fig. 43 - Acute inflammation, necrotizing, with hemorrhage, bladder (H&E).

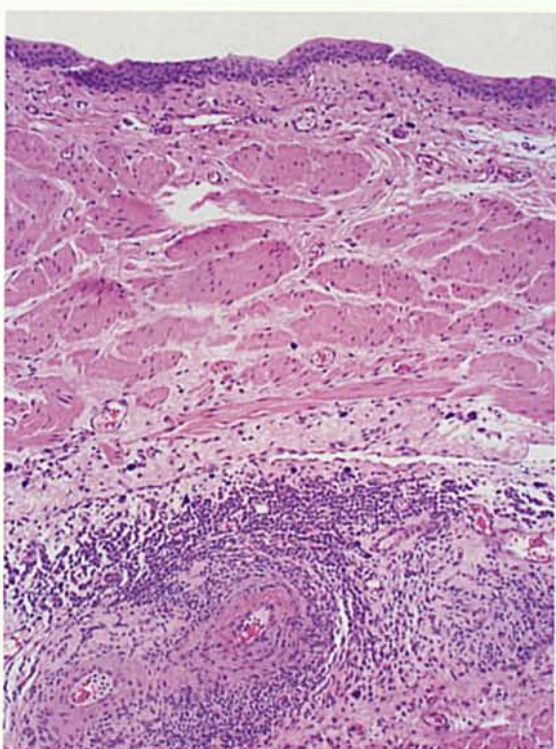


Fig. 44 - Chronic inflammation, bladder (H&E).

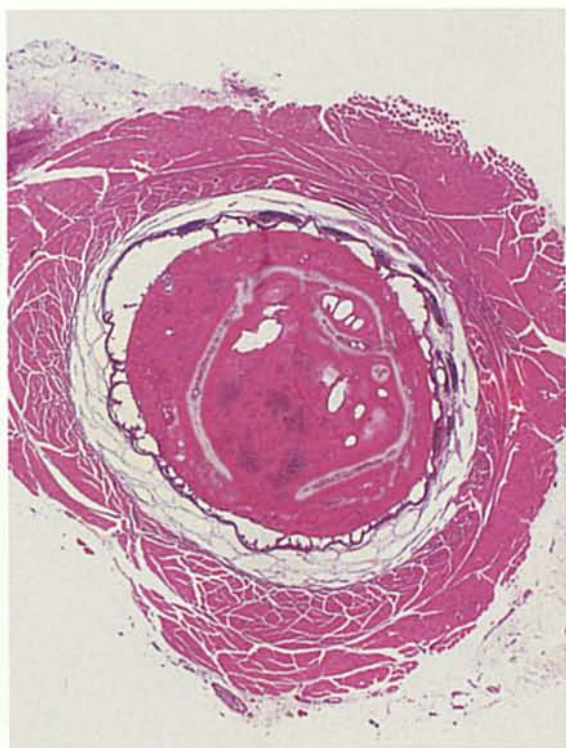


Fig. 45 - Proteinaceous plug, urethra (H&E).