Renal Biopsy and Pathologic Evaluation of Glomerular Disease

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Presence of suspected primary glomerular disease is the most common and compelling reason to consider renal biopsy. Pathologic findings in samples from animals with nephritic or nephrotic glomerulopathies, as well as from animals with persistent subclinical glomerular proteinuria that is not associated with advanced chronic kidney disease, frequently guide treatment decisions and inform prognosis when suitable specimens are obtained and examined appropriately. Ultrasound-guided needle biopsy techniques generally are satisfactory; however, other methods of locating or approaching the kidney, such as manual palpation (e.g., in cats), laparoscopy, or open surgery, also can be used. Visual assessment of the tissue content of needle biopsy samples to verify that they are renal cortex (i.e., contain glomeruli) as they are obtained is a key step that minimizes the submission of uninformative samples for examination. Adequate planning for a renal biopsy also requires prior procurement of the fixatives and preservatives needed to process and submit samples that will be suitable for electron microscopic examination and immunostaining, as well as for light microscopic evaluation. Finally, to be optimally informative, renal biopsy specimens must be processed by laboratories that routinely perform the required specialized examinations and then be evaluated by experienced veterinary nephropathologists. The pathologic findings must be carefully integrated with one another and with information derived from the clinical investigation of the patient's illness to formulate the correct diagnosis and most informative guidance for therapeutic management of the animal's glomerular disease.

Keywords: kidney, glomerular disease, renal biopsy, renal pathology, dog, cat, proteinuria

The purpose of renal biopsy is to obtain information that can help the clinician manage a patient's illness more astutely than might be possible without the biopsy and thus to obtain the best available outcome. This information might be the diagnosis of a particular nephropathic illness for which specific therapeutic options can be defined or refined. Additionally, and often independent of identifying a specific etiopathogenic diagnosis, renal biopsy typically yields information about the likely mechanism(s) of injury, as well as the severity, activity, chronicity, and/or potential reversibility of pathologic changes that are present, all of which support clinical decision-making about prognosis and treatment.[1-5]

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To achieve this purpose, 3 things must be true. First, the biopsy must be indicated; that is, it must be performed in a clinical situation in which the results of the biopsy will have potential utility. Second, the biopsy procedure itself (i.e., the process of obtaining adequate kidney tissue samples) must be done safely. Third, the tissue samples must be evaluated by individuals having expertise in nephropathology and using all the methods required to adequately characterize the important pathologic changes in the specimens and thus yield the most informative diagnosis. For suspected glomerular disease, which is the focus of this article, the required methods of evaluation are highly specialized and include special sectioning and staining protocols for the light microscopic evaluation, as well as routine use of transmission electron microscopic and immunostaining evaluations.

Indications

The presence of suspected glomerular disease is the most common and compelling reason to consider performing renal biopsy. Because proteinuria is a hallmark of glomerular injury, this particular laboratory test abnormality often is a key factor that prompts consideration of a renal biopsy. However, proteinuria has many possible causes and proteinuria in and of itself is not an appropriate indication for renal biopsy. The key point here is that it actually is the identification of a

proteinuric nephropathy (i.e., renal disease characterized a least in part by proteinuria) rather than the proteinuria per se that should prompt consideration of renal biopsy. A discussion of the proper assessment of proteinuria is beyond the scope of this article, but appropriate guidance is available elsewhere.[6]

Animals with proteinuric nephropathies for which renal biopsy should be considered usually are those that exhibit a nephritic or nephrotic glomerulopathy or have glomerular disease that is characterized by persistent subclinical renal proteinuria.[4,5] Many animals, especially dogs, with chronic kidney disease that has advanced to International Renal Interest Society stage IV or late stage III exhibit some proteinuria; however, biopsy of these animals usually is unrewarding and generally should be avoided.

Nephritic glomerulopathies are characterized by proteinuria that can have a wide range of magnitude but often is in the nephrotic range (arbitrarily defined as UPC > 3.5), with or without accompanying hypoalbuminemia that usually is of mild to moderate severity when it is present, and urinalysis findings that include signs of inflammation in the urinary tract (e.g., microscopic hematuria, mild pyuria). Most animals with nephritic glomerulopathies exhibit azotemia (the magnitude of which can be mild to severe) that typically also shows a rising trend in acute cases. Additionally, hypertension that frequently is severe and difficult to control medically often is present; however, edema or ascites is uncommon.

Animals with nephrotic glomerulopathies exhibit nephrotic range proteinuria associated with marked hypoalbuminemia that may or may not be associated with evident edema or third-space accumulation of transudates (e.g., ascites, pleural effusion). Animals with nephrotic glomerular diseases usually have totally inactive urine sediments and often do not have azotemia, especially early in the disease course. Hypertension is a variable feature of nephrotic glomerulopathies.

Animals with persistent subclinical renal proteinuria (proteinuria that is not of prerenal or postrenal origin and has been repeatedly documented over a period of a month or more in an animal that exhibits no related clinical signs) may have proteinuria of any magnitude, but it usually is of mild to moderate severity and associated with normal or only mildly decreased circulating albumin concentrations. These animals may or may not be azotemic. Indeed, this category overlaps with chronic kidney disease, especially in International Renal Interest Society stages I and II, and early stage III. These are among the most challenging animals in which to decide whether a biopsy will be useful. In general, the higher the UPC and the lower (i.e., more normal or near-normal) the serum creatinine concentration, the more a recommendation to biopsy can be supported, but each case should be considered individually instead of by applying any single UPC or serum creatinine cut off. In borderline cases, however, it is logical to be swayed toward biopsy by finding lack of response or worsening trends during treatment with nonspecific renoprotective interventions (i.e., feeding an appropriate diet, administering drugs to block portions of the renin-angiotensin-aldosterone system, etc.).

Figure 1. A mobile cart used at Texas A&M University to aid in the processing of renal biopsy specimens. The cart is readily taken to whatever site in the hospital (e.g., surgery or ultrasound suite, etc.) where specimen collection is planned. The top of the cart is prepared for processing a biopsy on-site. Note the dissecting microscope, which is used to assess specimen content. There also is an ice bucket to keep the fixative for electron microscopy chilled, as well as a thermos containing liquid nitrogen used to snap freeze samples for immunofluorescence microscopy after a shallow puddle of the liquid nitrogen is poured into the styrofoam freezing box.

Contraindications

Regardless of the indications for renal biopsy, it should not be performed (or should at least be delayed until the patient's condition is stabilized) if it cannot be performed safely. The main renal biopsy complication of clinical concern is serious hemorrhage.[7,8] Factors that are associated with increased risk of this complication are small patient size (i.e., small size of the biopsy target relative to adjacent large vessels), especially animals that weigh less than 5 kg, as well as the presence of disordered hemostasis (e.g., thrombocytopenia, prolonged bleeding time, etc.) or uncontrolled hypertension. Other relative or absolute contraindications to renal biopsy include inadequate control of patient pain or motion (including breathing), and insufficient operator competence.

Prebiopsy Planning

Adequate planning for a renal biopsy requires prior procurement of the materials needed to process and submit samples that will be suitable for the required examinations (Fig 1).

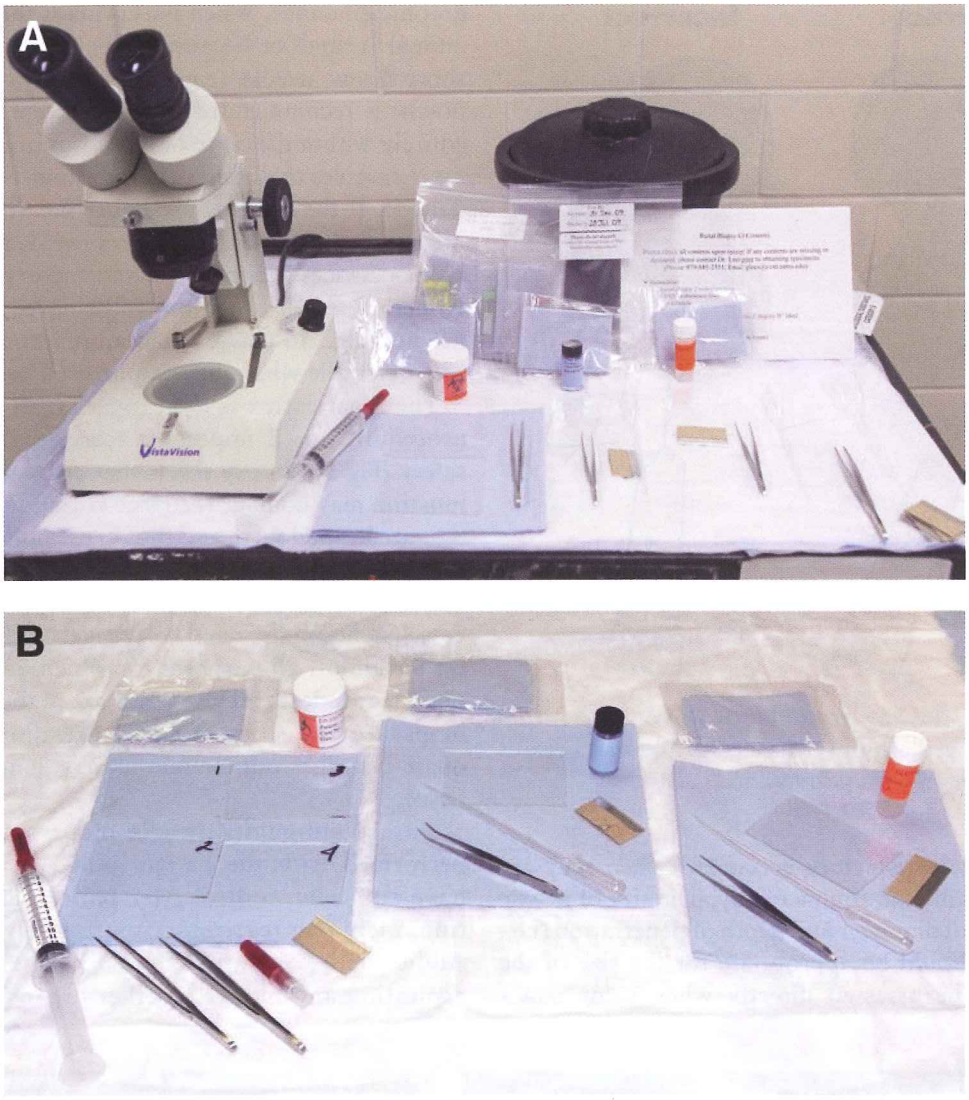

Figure 2. View of the top of the renal biopsy cart as prepared for processing specimens off-site utilizing the materials provided in renal biopsy kits that are available from centers that perform comprehensive pathologic evaluations (A) and a closer view of the contents of a renal biopsy kit prepared for use (B). There are appropriate fixatives for light microscopy (10% formalin) and transmission electron microscopy (3% glutaraldehyde), as well as a preservative (Michel's transport medium) for immunofluorescence microscopy. Tissue samples are placed on the glass slides for examination and kept moist with the saline solution in the syringe. The forceps have no teeth and are suitable for delicate manipulation of the samples. There are a sufficient number of forceps and single-edge razor blades to use separate instruments for specimens intended for each type of fixative or preservative to prevent cross-contamination. The pipettes are used to transfer fluids from the sample containers to the surface of the glass slides and then to wash the samples off the glass slides into their respective specimen containers. Reprinted with permission.[9]

For glomerular disorders, light microscopic examination alone is not sufficient. The samples needed for electron microscopic examination and immunostaining must be appropriately processed and placed into the proper fixatives and preservatives when the tissue specimens are first obtained. Centers that perform these evaluations provide kits containing the materials and instructions needed to obtain, process, and submit satisfactory renal biopsy specimens to their laboratories (Fig 2).

Biopsy Specimen Acquisition

Performing a renal biopsy requires selection of a suitable method of approaching or locating the target kidney and choice of a device or method for retrieving the tissue.[9] Ultrasound-guided needle biopsy techniques are commonly used and generally are satisfactory when the expected changes are likely to be diffusely distributed in the cortex, as is the case for most glomerular disorders. A variety of automated biopsy

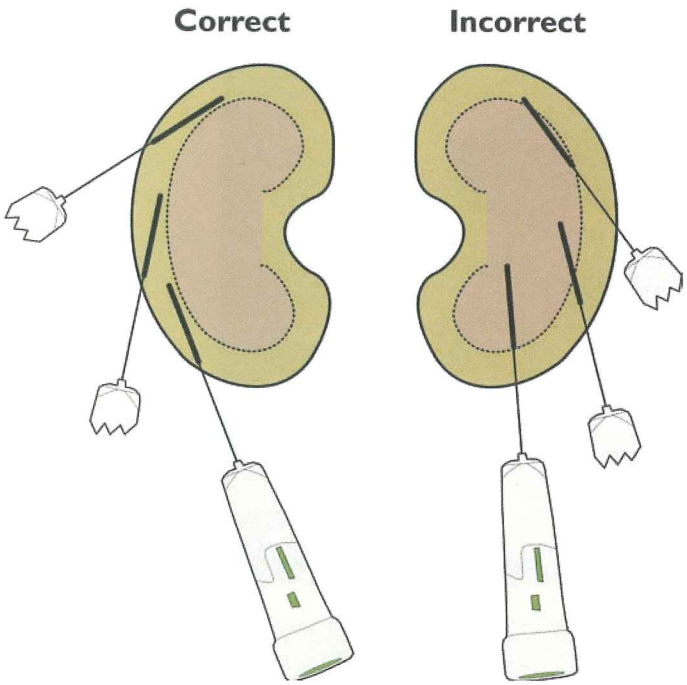

Figure 3. Diagram showing examples of correct and incorrect placement of needle biopsy devices to obtain cores of renal cortex. Reprinted with permission.[9]

devices that procure satisfactory specimens when they are used properly are available commercially. The size of needle used, both in terms of diameter and depth of penetration (i.e., length of throw), should be appropriate for the size of the target, which can be assessed directly when using sonographic guidance. When the cortex is thin either because the animal is small or because of the effects of disease, use of a short-throw needle (e.g., 11-mm throw; 7-mm specimen notch) is recommended to aid in keeping the biopsy tracts entirely within the cortex even if it is necessary to take a few more samples to obtain sufficient tissue for all intended evaluations. If the samples are intact (i.e., not fragmented) and are handled carefully, the cores provided by 18-gauge needle biopsy devices generally are satisfactory for most purposes. Nonetheless, all other things being equal, larger-diameter needles (e.g., 16 gauge) yield more informative samples and are preferable when they can be used safely.

The renal cortex is the proper target for all renal biopsy procedures for 2 important reasons, the first of which is safety (Fig 3). Biopsy tracts that cross the corticomedullary junction may damage the large vessels (e.g., arcuate arteries) that are located there and thus cause both excess hemorrhage and greater damage to the renal parenchyma as a result of ischemia or infarction of the region(s) served by the damaged vessel(s). Secondly, renal cortex is the primary tissue of interest for almost all purposes for which biopsies of kidney are obtained. Indeed, all glomeruli are in the cortex, and a renal biopsy for evaluation of glomerular disease is wholly inadequate if it does not contain an adequate sample of cortical tissue.

Ultrasound-guided needle biopsy of kidney should be performed with the patient under general anesthesia to have sufficient control over patient discomfort and motion, including respiratory motion, during the procedure. Additionally, operators should have extensive practice in aspiration and biopsy of other organs and masses before

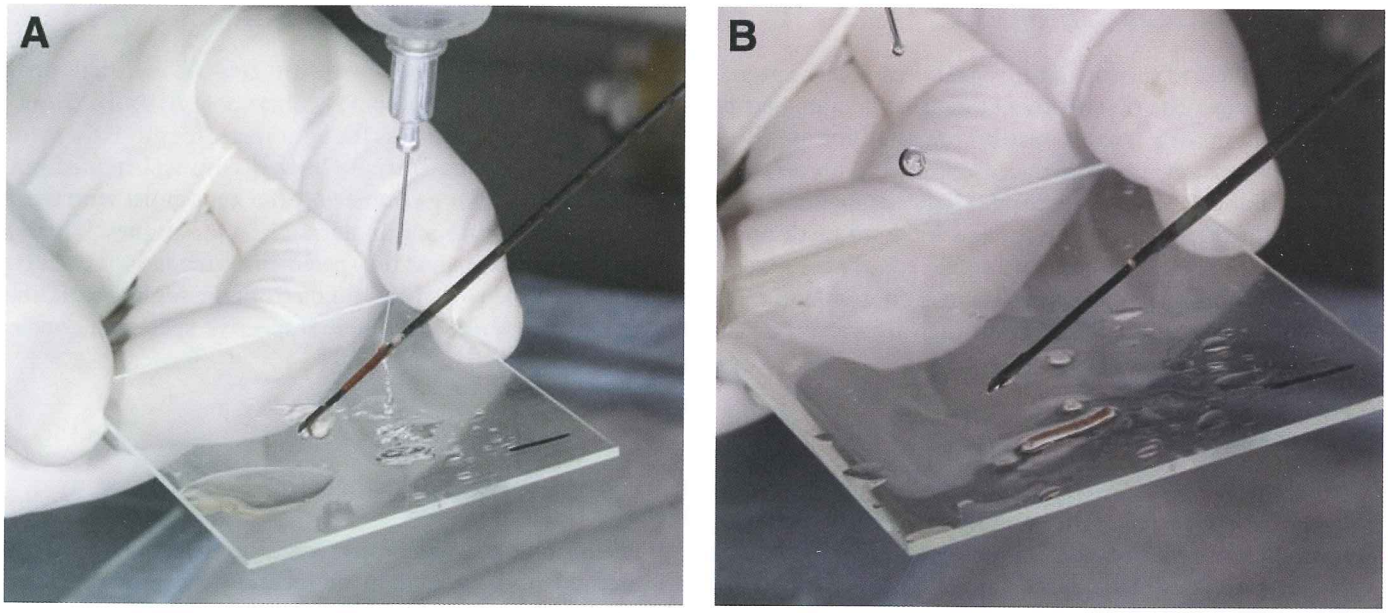

Figure 4. Method for retrieving a renal tissue core from a needle biopsy device using gentle flow of sterilized normal saline solution through a 25 gauge needle (A) to wash the specimen onto the surface of a glass slide (B) without permitting the sterile biopsy needle to touch the glass slide. After the specimen is retrieved, vigorous flow of saline can be used to dislodge any tissue tags that remain in the specimen notch before re-using the biopsy needle to obtain an additional core. Reprinted with permission.[9]

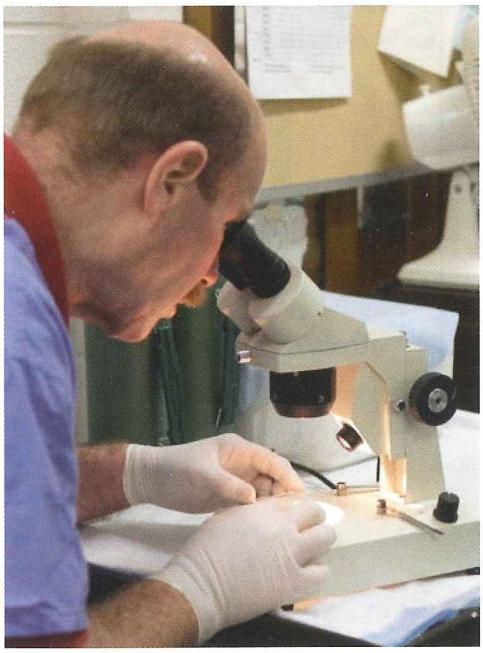

Figure 5. Use of a dissecting microscope with 20X to 40X magnification to assess the tissue composition of each biopsy core that is retrieved. Reprinted with permission.[9]

attempting kidney biopsy because there is very little margin for error. There are several different combinations of patient positions, scanning angles, needle directions, and aspects of the kidney to be the biopsy target(s) that can be used successfully depending on operator preferences. The primary concern is that the choice of approach should minimize the possibility of inadvertent damage to the major renal vessels in the hilus because this can lead to a catastrophic outcome (e.g., fatal hemorrhage). Some prefer to biopsy the right kidney because it typically is less mobile (i.e., because it is held in place against the liver) than the left kidney; however, others prefer the left kidney because of its more caudal and superficial location. A biopsy guide attached to the scanning probe to direct the needle biopsy device is the safest method to use, but some people prefer to use a "free hand" technique in which the probe and biopsy device are not connected to one another and can be manipulated independently until the operator is certain that the needle is positioned optimally. This latter method requires excellent hand-eye coordination and extensive operator experience. Although a scan plane that includes both cortex and medulla often makes the kidney easy to recognize, a scan plane that includes only cortex (in which the biopsy tract will be confined) is recommended for renal biopsy. The lateral cortex (if imaging in a ventrodorsal position) or the dorsal cortex (if imaging in a lateral position) often is the best area from which to obtain samples based on the availability of sonographic windows and ability to position the biopsy device appropriately. These areas also are distant from the hilus, which minimizes the risk of damaging the major vessels located there.

The kidney typically is visualized in a sagittal or dorsal plane after which the operator should fan the scan plane so that only cortex remains in the plane in which the biopsy needle will be placed. When the scan plane and direction of needle placement have been identified, a small stab incision is made through the skin at the entry point to minimize dulling of the biopsy needle before it is advanced through the body wall to the kidney. In addition, and before activation of the biopsy device, it often is necessary to advance the biopsy needle tip into the capsule of the kidney (especially when biopsying the left kidney) to min-

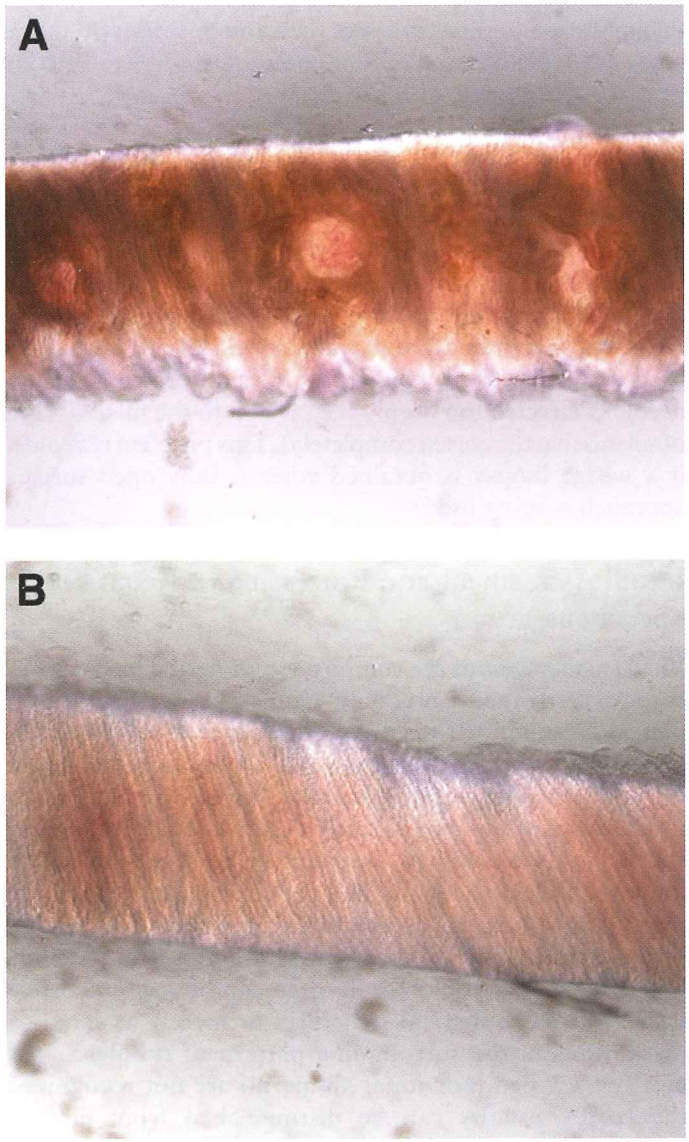

Figure 6. Photomicrographs of renal biopsy cores showing the difference in appearance of cortex (A) and medulla (B). Several glomeruli (the spherical structures and spherical disruptions in the surrounding pattern of tubules) are visible in the cortical specimen (magnification 40X). Reprinted with permission.[9]

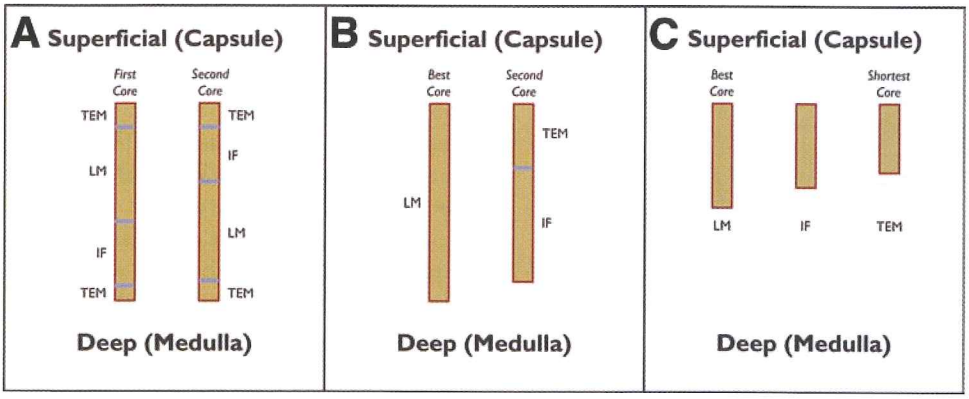

Figure 7. Diagrams showing recommended ways to subdivide renal biopsy cores for light microscopic (LM), transmission electron microscopic (TEM), and immunofluorescence (IF) evaluations when: (A) the cores are not examined with sufficient magnification to directly assess their composition; (B) the cores are verified to be composed of cortex and 2 long (> 10 mm) cores are available; and (C) the cores are verified to be composed of cortex and 3 short (< 10 mm) or fragmented cores are available. Reprinted with permission.[9]

imize movement of the kidney away from the biopsy instrument when it is activated.

Needle biopsy devices also can be used in combination with other aiming methods including manual palpation (e.g., in cats), laparoscopy, and a keyhole or fully open celiotomy. However, a common pitfall when needle biopsy devices are used to obtain a kidney biopsy during a celiotomy is that the needle is directed too deeply (i.e., well into the medulla, and often missing the cortex completely). This problem is avoided if a wedge biopsy is obtained when a fully open surgical approach is being used.

Initial Assessment and Processing of Renal Biopsy Specimens

Visual assessment of the composition of needle biopsy tissue cores to verify that they contain glomeruli (i.e., that the samples to be submitted for evaluation are cortical tissue) is a crucial step when performing a renal biopsy—and one that often is overlooked (Figs 4 and 5). This task is best accomplished with a low level of magnification (10-40×), such as can be achieved with a dissecting microscope, an ocular loupe, or a handheld lens, and good illumination. With such magnification, several aspects of the appearance of core biopsy specimens aid differentiation of cortex from medulla (Fig 6). One is that glomeruli often can be seen in cortical tissue as small spherical structures or merely as spherical disruptions in the surrounding pattern of tubules. However, even when individual glomeruli are not recognized, the cortex usually can be distinguished from medulla based on the general architecture of the tissue because tubules in cortical tissue are convoluted (they appear jumbled in an irregular pattern), whereas those in medulla are straight and arrayed in parallel with one another. Additionally, the specimens should be kept moist (i.e., never placed on dry sponges) with physiologic saline solution and manipulated with great care (i.e., very gently and without grasping them with forceps) as they are collected, assessed, processed, and placed in appropriate fixatives or preservatives.

In general, it is best to collect at least 2 cortical cores if each is >10 mm long (Figs 7 and 8). When the cores are shorter than 10 mm each, 3 cores usually are required. Needle biopsy cores do not need to be cut into smaller pieces except as needed to subdivide them for separate evaluations; however, a portion of a wedge biopsy specimen must be carefully cut into pieces that are no more than 1 to 2 mm in any dimension before they are put in the fixative for electron microscopy (Fig 9).

Pathologic Evaluations

Nephropathology is unique among the subspecialties of anatomic pathology for its routine use of ultrastructural (transmission electron microscopic) examinations together with histologic (light microscopic) findings and immunostaining (immunofluorescence microscopy) for the diagnosis of common diseases, especially those affecting glomeruli.[10] Moreover, even the light microscopic evaluation of renal biopsy specimens requires the routine use of special techniques and stains to reliably demonstrate the pathologic changes that need to be assessed. And finally, all of the pathologic findings must be carefully integrated with one another and with information derived from the clinical investigation of the patient's illness to formulate the correct diagnosis and most usefully inform the patient's clinician about therapeutic options and prognosis.

Light Microscopic Evaluation

Performing an optimally informative light microscopic evaluation of a renal biopsy sample begins with special considerations during the processes of embedding and sectioning the tissue. Kidney samples, especially small-diam-

Figure 8. Division of a needle biopsy core (A) with a clean razor blade (B) into 2 portions (C) for separate evaluations (e.g., one to be processed for transmission electron microscopic evaluation and the other to be processed for immunofluorescence microscopic evaluation) after examination with sufficient magnification to verify that the core is composed entirely of cortical tissue. Reprinted with permission.[9]

eter (e.g., 16, 18 gauge) needle biopsy cores, are fragile and easily damaged or distorted by indiscriminant handling. Thus, laboratory personnel must take special precautions to avoid creating artifacts when processing and embedding

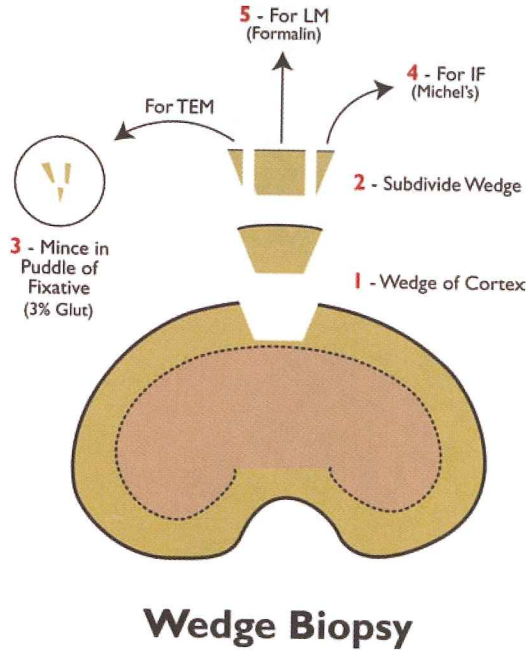

Figure 9. Diagram showing the recommended steps to subdivide and process a wedge biopsy of kidney for light microscopic (LM), transmission electron microscopic (TEM), and immunofluorescence (IF) evaluations, including: (1) retrieve a wedge of cortex from the kidney; (2) subdivide the wedge by trimming small portions from each side for the TEM and IF evaluations; (3) transfer the portion for TEM evaluation into a puddle of chilled TEM fixative (e.g., 3% glutaraldehyde) on a glass slide and cut into smaller pieces; (4) put the portion for IF evaluation in an appropriate preservative (e.g., Michel's transport media); and (5) put the portion for LM evaluation in an appropriate fixative (e.g., 10% buffered formalin). Reprinted with permission.[9]

such samples. Additionally, highly specialized sectioning protocols are required. The first issue is that renal tissue sections must be cut thinner (i.e., 2-3 μm thick) than sections of other tissues usually are cut for routine histopathology (i.e., at 5-6 μm thick). Examination of appropriately thin sections is crucial especially for assessment of glomerular cellularity, as well as for assessment of the glomerular basement membranes using special stains. The next issue is that sections must be cut and mounted on multiple glass slides because multiple different stains are routinely used to evaluate the kidney. In the laboratories we direct, we routinely use at least 5 different stains to evaluate the kidney, and in some circumstances the additional use of several other special stains is crucial. And lastly, it is important that a sufficient number of serial sections be cut and mounted on slides for the various stains so that adjacent sections, as stained by the different methods, can be compared, as well as so that the changes present at multiple "levels" (i.e., different planes of sectioning) within the sample can be compared.

The importance of routine use of the special stains needed to properly evaluate the kidney cannot be overemphasized (Fig 10). Hematoxylin and eosin--stained sections are very useful, especially for assessing the cells in the sections (i.e., the intrinsic cellular components of glomeruli, tubules, interstitium, and vessels, as well as extrinsic cells, such as in infiltrates of inflammatory cells). However, the hematoxylin and eosin stain does not provide the essential information about the extracellular components of kidneys (e.g., mesangial matrix, glomerular and tubular basement membranes, Bowman's capsule, interstitial matrix, etc.) that is required to make adequately informed light microscopic assessments of (diagnoses for) kidney diseases. The special stains that are most commonly used routinely are periodic acid Schiff (PAS), Jones methenamine silver, and Masson's trichrome. The PAS stain, which usually is used with a hematoxylin counterstain (i.e., PASH) for kidney, accentuates the architecture of the tissue because it highlights the borders between tissue compartments (i.e., the tubu-

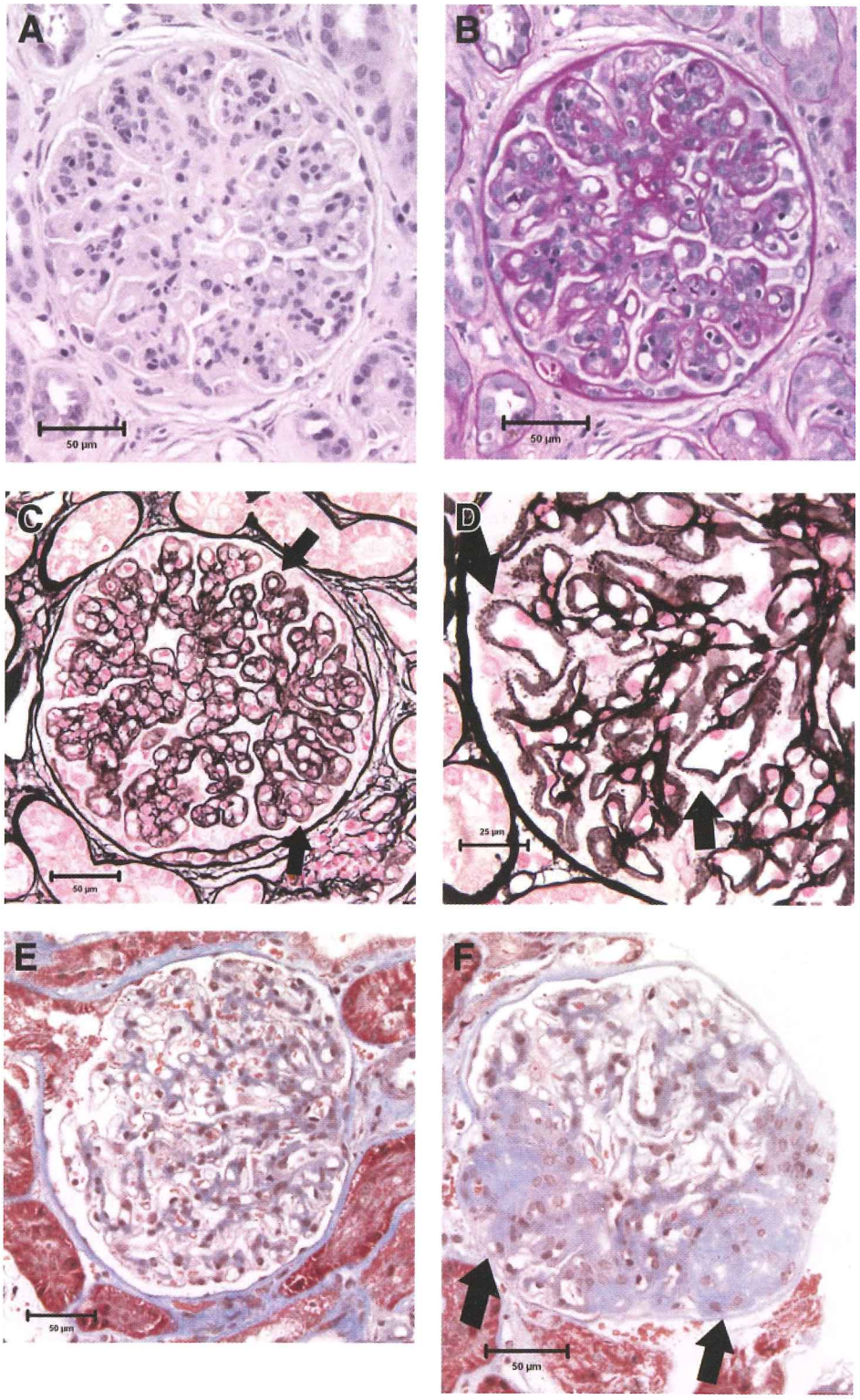

Figure 10. Photomicrographs illustrating the value of stains routinely used to evaluate glomerular lesions. Panels A and B show membranoproliferative GN; the PAS stain (B) does a better job of delineating the different structures within the glomerulus than does the H&E stain (A). (C) and (D) are Jones methenamine sliver stains that show changes in the GBM; many capillary walls in the glomerulus in panel C exhibit double GBM contours (arrows), which are indicative of mesangial cell interpositioning, whereas the GBM in many capillary walls in the glomerulus in panel D exhibit spikes and holes (arrows) that are typical of membranous GN. (E) and (F) show Masson's trichrome stained glomeruli from a dog with glomerulosclerosis that has a focal and segmental pattern of distribution; that is, the lesion is present in some but not all glomeruli (absent in E, present in F), and is present in some (arrows) but not all lobules within affected glomeruli (F).

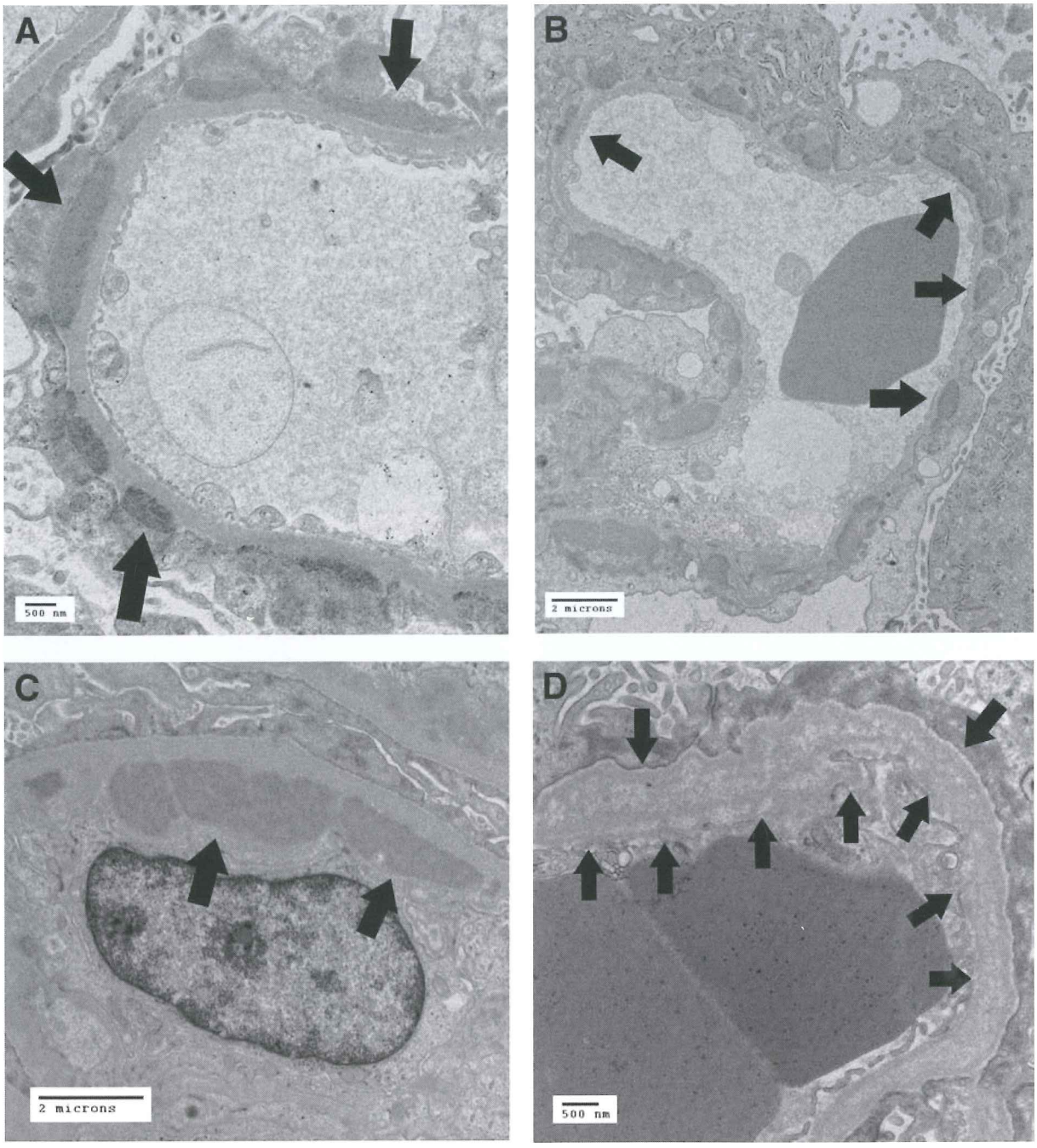

Figure 11. Photomicrographs of examples of transmission electron microscopic findings for several types of glomerular disease. (A) and (B) show subepithelial electron-dense (immune-complex) deposits (arrows) that are characteristic of membranous glomerulonephropathy. In panel A, there is minimal remodeling of the glomerular basement membrane (GBM) in response to the deposits; whereas, in panel B, most of the deposits are surrounded by new GBM matrix. (C) shows subendothelial electron-dense (immune-complex) deposits (arrows) that are characteristic of membranoproliferative glomerulonephritis. (D) shows the changes in GBM ultrastructure that are characteristic of inherited disorders of type IV collagen (canine hereditary nephropathy); the GBM (between the arrows) is thickened and exhibits multilaminar splitting.

lar, Bowmen's capsule, and glomerular capillary wall basement membranes) and also because it stains various other substances in informative ways. The Jones methenamine silver stain is particularly useful for assessing the fine structure of the glomerular basement membranes (e.g., thickness, irregularity, presence of "spikes," holes, double contours) at the light microscopic level. The trichrome stain, which stains collagen blue, is particularly useful for the assessment of fibrosis (e.g., interstitial fibrosis, glomerulosclerosis), but like the PAS stain, also stains various other substances and structures in

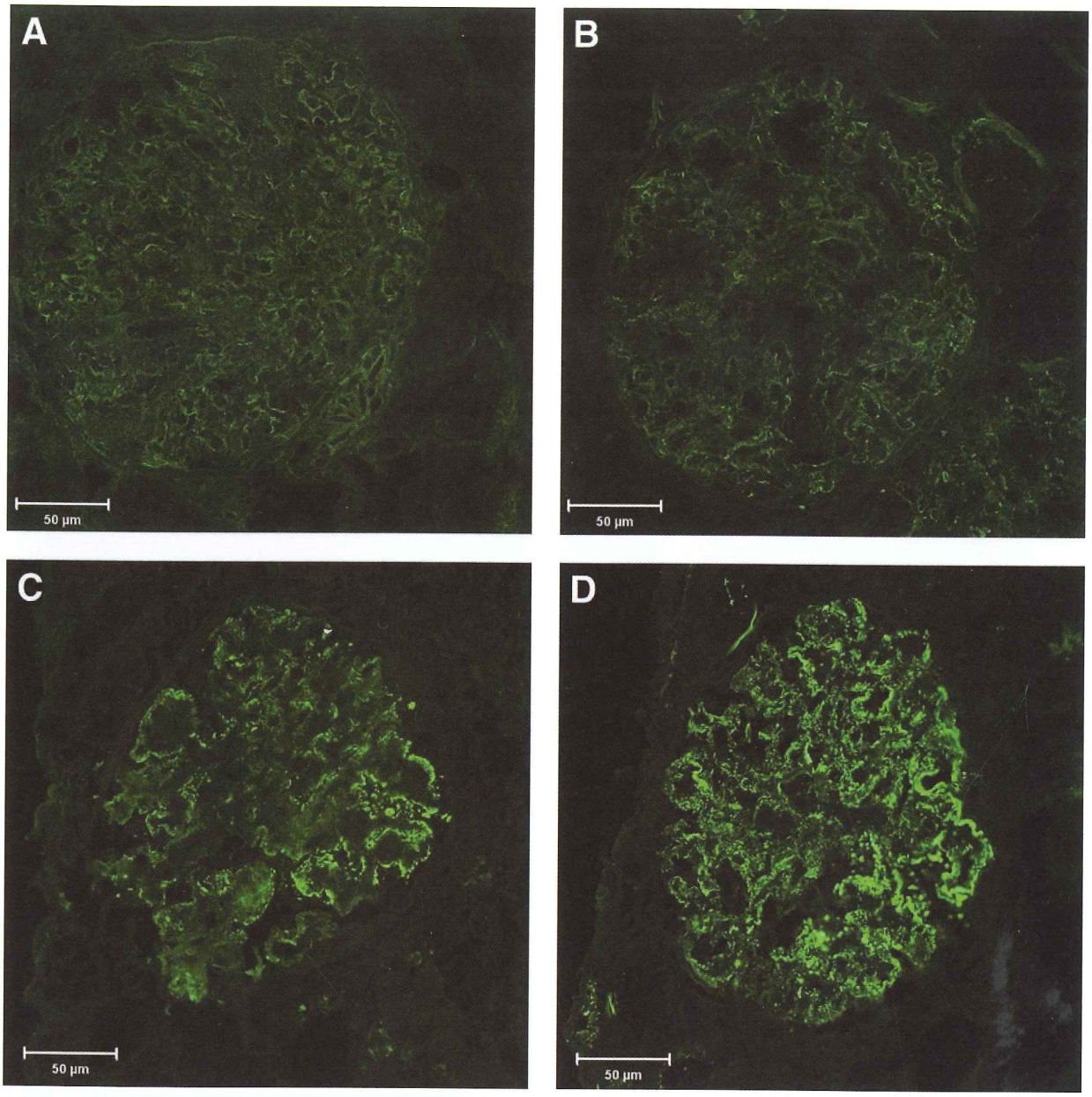

Figure 12. Fluorescence photomicrographs of glomeruli labeled for immunoglobulin G (IgG; panels A and C) and the 3rd component of complement (C3; panels B and D). (A) and (B) show the findings from a dog with membranous glomerulonephropathy, and (C) and (D) show findings from a dog with membranoproliferative glomerulonephritis.

informative ways. Beyond these, the next most commonly used stain is Congo Red (for amyloid); however, there is a long list of other special stains that are useful in various circumstances.

Transmission Electron Microscopic Evaluation

Because of the laws of physics, the best resolution that can be obtained with optical (light) microscopy is that provided by about 1000× magnification (e.g., a 100× oil immersion objective lens and a 10× ocular lens). However, with transmission electron microscopy, it is possible to discern morphologic features of structures that are much smaller than can be seen with optical microscopy. Indeed, with electron microscopy, 1000× is a very low level of magnification.

Many renal diseases, especially glomerular diseases, are defined pathologically at least partly by distinctive morphologic changes in renal cells and/or extracellular matrixes that can only be detected with electron microscopy (Fig 11). Some of the more important ultrastructural changes that are identified by electron microscopy are abnormalities in the component layers of the glomerular capillary walls; that is, endothelial cells, glomerular basement membrane, and visceral epithelial cells (i.e., podocytes). Additionally, electron microscopy is used to detect and char-

acterize the distinctive features of various kinds of organized deposits in glomeruli. Chief among these are electron-dense deposits of immune complexes, which have different implications, and identify different disease processes, depending on the location(s) where they are found (i.e., in mesangium and/or in capillary walls, where their locations may be further categorized as being subepithelial, intramembranous, subendothelial, or associated with mesangial cell interpositioning). Another albeit less common type of organized glomerular deposit that requires ultrastructural evaluation for its diagnosis is the material that characterizes nonamyloidotic fibrillary glomerulopathy.

Immunostaining

Immunostaining methods use antibodies that bind to specific epitopes to identify the presence or absence and, if present, the location, of entities (molecules or structures containing molecules) that are of particular interest within the tissue. In nephropathology, the most common use of this technique is to determine presence or absence and, if present, the distribution of, various immune reactants (e.g., immunoglobulin [Ig] G, IgM, IgA, C3, etc.) in glomeruli (Fig 12). Conceptually, however, immunostaining can be used to probe for the presence and location of any entity that the searcher wants to find, if the searcher has an antibody that will bind to that entity in a specific fashion. Additionally, after the probe antibody has bound to the epitope of interest, the searcher has to detect the presence of the probe, so any antibody that is used for this purpose has to be "labeled" in some way that permits it to be detected. The most common way this is done in diagnostic nephropathology is with the use of antibody probes that are tagged with fluorescent labels (e.g., fluorescein isothiocyanate) and to then use an epifluorescent microscope fitted with appropriate filters to examine the specimens.

The most useful type of renal biopsy specimen to use for immunostaining is tissue that has not been fixed because fixation (such as by formalin) causes proteins that are merely present in the circulation (i.e., in the plasma that permeates the tissue) to become bound within the specimen. Immunostaining such specimens typically results in excess background staining; that is, proteins from the plasma (e.g., IgG) are labeled along with any pathogenic deposits of the same proteins (e.g., IgG in deposits of immune complexes) that may have been present in the tissue in vivo. This makes it difficult to impossible to confidently differentiate labeling that is indicative of a disease process from labeling that is merely background staining. The preferred method is to instead use cryosections (i.e., frozen sections) cut from unfixed tissue for immunostaining. Sections cut from fresh tissue that was properly snap-frozen either immediately or after up to several (3 to 5) days of storage (e.g., during shipping to the laboratory) in an appropriate preservative (not fixative), such as Michel's transport medium, are mounted on microscope slides and then washed. When this is done, proteins that were not already bound within tissue when the sample was first obtained (i.e., plasma proteins) are washed away, and the evaluation of immunolabeling using such sections is confounded by little or no background staining.

Especially in human medicine, many specific renal disease entities are defined pathologically (at least in part) by the immunostaining findings (composition and location of immune reactants) that are exhibited. Moreover, other diseases are defined (again, at least in part) by the absence of reactivity for (certain) immune reactants. Equivalent information is not well established for renal diseases of dogs and cats; however, helpful data are beginning to emerge from ongoing studies. Moreover, observations about the presence or absence of reactivity for immune reactants, as well as the labeling pattern that is exhibited when they are present, can provide insights regarding the pathogenesis of incompletely understood or newly recognized diseases as they are encountered.

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