Proteinuria

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The normal glomerulus is a highly selective barrier for filtration based on size (and on charge in the case of larger molecules). While small dissolved particles are freely filtered, larger molecules are almost excluded (Table 1).

Table 1. Glomerular filtration of dissolved substances based on size

	M.W.	S-E Radius	Filtrate/ Plasma
Inulin	5300	1.4	1.0
Insulin	6000	1.6	0.9
Lysozyme	14,600	1.9	0.75
Myoglobin	16,900	1.9	0.75
PTH	9,000	2.1	0.65
Growth Hormone	20,000	2.1	0.6-0.7
Amylase	48,000	2.9	0.02
Albumin	69,000	3.6	0.02
Gamma Globulin	160,000	5.5	0.00
Ferritin	480,000	6.1	0.02

Under normal circumstances, only a small proportion of plasma albumin and almost no plasma globulins cross the glomerular filtration barrier to enter Bowman's space and the renal tubule. Of the filtered protein, almost all is reabsorbed in the proximal convoluted tubule and only very small quantities are observed in the final urine. Proximal tubular reabsorption of albumin and other proteins is by inclusion into apical lysosomal vesicles which move into the cytoplasm wherein proteins undergo metabolic breakdown. Reabsorption of albumin in the proximal convoluted tubule is normally at or near the transport maximum so increased albuminuria may be observed either if there is an increase in the filtered load of protein

(glomerular dysfunction) or with a decrease of tubular reabsorption of protein (tubular disease). Glomerular proteinuria can be minor to massive while tubular proteinuria is relatively minor.

Measurement and Assessment of Proteinuria

Methods commonly used to measure protein levels in urine include:

1. Microalbuminuria (MA) test. The test is specific for albumin, corrects for urine specific gravity and is accurate for levels between 1-30 mg/dl.
2. Urine dipstick test: The test detects albumin levels as low as 30 mg/dl. Excessively alkaline and highly concentrated urine can cause false positive results. Results should be interpreted with simultaneous consideration of the urine specific gravity.
3. Urinary sulfosalicylic acid turbidometric test: Confirms dipstick findings and eliminates the false positive reactions that such tests often produce.
4. Urine protein/creatinine ratio (UPC): This test is performed on a single spot urine sample and values are proportional to 24-hour urine excretion of protein. The Biuret method is inappropriate for estimation of urine protein because concentrations are so low. The Coumassie Blue test and Trichloro-acetic acids tests are accurate.

Hematuria and urinary tract infection elevate urinary protein levels. Such conditions should be corrected before attempting to interpret proteinuria as a reflection of glomerular disease.

Prior to the introduction of the MA test, expected normal values for protein in urine were: Dipstick: Zero to trace (corresponding to < 30 mg/dl); UPC: < 1 (corresponding to < 30 mg/kg/day). Now with the MA test capable of identifying patients with urinary albumin levels between 1-30 mg/dl, new values for normal need to be defined. At this point it is not yet clear to what extent "normal" animals may be microalbuminuric. The presence of MA may be significant or of no consequence with respect to development and progression of renal disease. One survey indicated the presence of MA in an increased proportion of older dogs.

Once the presence of proteinuria has been confirmed consideration should be given to determination of the source, magnitude and persistence (Table 2). Heavy proteinuria usually indicates serious disease.

Table 2. Clinical classification of proteinuria

- Prerenal: Plasma protein abnormality, e.g., Bence-Jones proteinuria
- Renal:
    - Functional: Transient, e.g., extreme exercise
    - Pathological:
        - Glomerular: Increased capillary wall permeability
        - Tubular: Decreased tubular reabsorption of protein
        - Interstitial: Exudate from peritubular capillary
- Postrenal:
    - Urinary: Exudate from lower urinary tract, e.g., UTI
    - Extraurinary: Genital exudations

Causes and mediators of glomerular injury

Glomerular proteinuria is caused by decreased selectivity of the glomerular filtration barrier so that large proteins are filtered from the plasma into the renal tubular. This may be due to generalized vasculopathies, specific accumulation of antigen-antibody complexes in various locations in the glomerulus, or amyloidosis (Table 3). Tubular proteinuria has been identified in human medicine and may occur in veterinary medicine but as yet this phenomenon has yet to be well defined in small animal clinical patients.

Although proteinuria is a common phenomenon, in most instances the glomerulus is affected secondary to disease processes occurring elsewhere in the body. The filtration function of the glomerulus renders it uniquely susceptible to injury and many "non-renal" diseases induce proteinuria (Table 3). Often, the primary disease process is self-limiting, the degree of proteinuria is mild and transient, and there are few or limited consequences for long-term renal function.

Chronic inflammatory conditions with mild antigen excess promote development of circulating antigen-antibody complexes that deposit in the glomerular basement membrane (GBM). Alternatively, free antigens can deposit in the GBM and antibodies can bind to form the antigen-antibody complex *in situ*. Antigen-antibody complexes activate complement, which induces infiltration of neutrophils and macrophages and initiates a complex array of inflammatory mediators and growth factors that disrupt normal glomerular structure and function.

Table 3. Diseases associated with proteinuria

DOG	CAT
Infectious	**Infectious**
Bacterial endocarditis	Feline leukemia virus
Brucellosis	Feline infectious peritonitis
Dirofilariasis	Mycoplasma polyarthritis
Ehrlichiosis	
Leishmaniasis	
Pyometra	
Borreliosis	
Chronic bacterial infection	
Rocky Mountain spotted fever	
Septicemia	
Neoplastic **Inflammatory**	**Neoplastic** **Inflammatory**
Pancreatitis	Pancreatitis
Systemic lupus erythematosus	Systemic lupus erythematosus
Polyarthritis	Other immune-mediated dis.
Prostatitis	Chronic skin disease
Inflammatory bowel disease	
Immune mediated hemolytic anemia	
Other immune-mediated dis.	
Sulfonamide hypersensitivity	
Other	**Other**
Idiopathic	Idiopathic
Hyperadrenocorticism	Amyloidosis

Amyloidosis	Chronic kidney disease
Chronic kidney disease	
Inherited nephropathies	
IgA nephropathy	
Soft coated Wheaton terrier nephropathy	

In a small proportion of animals with glomerulonephritis and in most dogs with glomerular amyloidosis, glomerular injury and urinary protein loss is so massive that it leads to hypoalbuminemia, edema and ascites. The combination of heavy proteinuria, hypoproteinemia and peripheral edema is known as the nephrotic syndrome. Severe, unrelenting GN reduces GFR with eventual development of chronic kidney disease (CKD). Dogs with severe proteinuria develop hypercholesterolemia, hyperfibrinogenemia, hypertension, and hypercoagulopathy due to renal loss of antithrombin III.

Role of proteinuria in the progression of chronic kidney disease

There is evidence that proteinuria may be involved in the progression of CKD. In human patients with diabetes mellitus and in patients with hypertension, development of microalbuminuria is a sentinel predicting the onset of CKD. Similar phenomena have been observed in dogs with IgA nephropathy and in soft-coated Wheaton terriers with familial renal disease where appearance of MA portends overt renal disease. In separate studies, azotemic dogs and cats with higher levels of proteinuria on first presentation had reduced survival.

In addition, in humans, the appearance of MA has been associated with increased risk of myocardial infarction in patients with other major risk factors. One study in non-azotemic cats indicated decreased survival from all causes when the UPC exceeded 0.43.

Emerging evidence implicates breakdown products of reabsorbed tubular protein in the progression of renal damage in CKD. Renal tubules respond to proteinuria by increasing reabsorption of filtered protein. Filtered antibodies may attach directly to the tubular epithelial cells inducing injury. Filtered proteins and lipids that undergo tubular reabsorption are metabolized to toxic substances that induce interstitial inflammation and fibrosis.

Diagnosis

Microalbuminuria may be observed in dogs under a wide variety of conditions including vasculopathies, immune responses to non-renal disease, hemodynamic effects (corticosteroid administration), and renal disease. The presence of microalbuminuria may be an early diagnostic marker for serious occult renal and non-renal disease or may be a transient incidental finding associated with an already identified condition. If MA is detected in 2-3 consecutive tests in an otherwise normal appearing dog, non-renal conditions known to induce microalbuminuria should be ruled out before concluding that intrinsic renal disease may be the cause of altered glomerular permselectivity.

The hallmark of diagnosis in GN is quantitation of 24-hour protein excretion. Daily protein excretion can be measured in a 24-hour urine collection or estimated from the urine protein to creatinine ratio of a single sample of urine according to the following equation: 24 hr Urine Protein (mg/kg/day) = $U_{Pr/Cr}$. x 30.

In practice, urine protein concentration (mg/dl) is divided by urine creatinine concentration (mg/dl). In normal animals $U_{Pr/Cr}$ < 1 is normal, between 1 and 3 is questionable and greater than 3 is consistent with GN. Dogs with nephrotic syndrome usually spill more than 200 mg/kg/day of protein in the urine so have a $U_{Pr/Cr}$.> 6.

Once glomerulonephropathy is recognized, a diligent search for the underlying cause should be undertaken. An antinuclear antibody test for systemic lupus erythematosus, tests for dirofilariasis, and thoracic and abdominal radiographs for pyometra, abscesses, and

neoplastic processes should be performed.

The nature of glomerular injury can be characterized by examination of biopsy specimens under light microscopy. Staining with H & E and silver stains can provide an indication of cellular morphology of the lesion, thickening of the GBM, and the extent of glomerulosclerosis. Examination of Congo Red stained tissues under polarized light allows confirmation of amyloidosis. Immunofluorescence using antibodies directed against complement and immunoglobulins can establish whether glomerular injury is due to immune-complex deposition. Electron microscopy provides further definition of the location of antigen-antibody deposits.

Table 4. Management of heavy proteinuria

- Immunosuppressive agents
  - Azathioprine
  - Corticosteroids
  - Cyclophosphamide
- Inhibitors of the inflammatory processes
  - Specific thromboxane inhibitor
- Dietary adjustment
  - Reduced protein
  - High N3:N6 polyunsaturated fatty acid ratio
- Vasoactive agents
  - Angiotensin converting enzyme inhibitors
  - Antihypertensive agents
- Anticoagulants
  - Aspirin
- Diuretics

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