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Renal structure and physiology

The kidney is an organ with endocrine properties, and the capacity to synthesise and catabolise proteins. However, its fundamental role is to remove fluid and potentially toxic substances by the production of urine as an ultrafiltrate of plasma, and to maintain homeostasis of body protein. These latter functions are the task of the nephron, a microscopic unit comprising the glomerulus, Bowman's capsule, proximal and distal convoluted tubules, and the collecting ducts (Fig. 1.1). The various components of the nephron interact with the systemic circulation, thereby influencing renal handling of plasma proteins. Before discussing this in detail, the structure of the nephron components will be summarised briefly.

The glomerulus is a capillary network composed of a thin layer of endothelial cells, a central region of mesangial cells with surrounding mesangial matrix material, visceral epithelial cells with associated basement membrane, and the parietal epithelial cells of Bowman's capsule with its basement membrane. Bowman's (urinary) space lies between these two epithelial layers, and afferent and efferent arterioles control the capillary blood flow (Fig. 1.1). Glomeruli are innervated by autonomic nerves, and neural control may be particularly important in the larger juxtamedullary glomeruli, close to which is the site of renin secretion.

Molecules in the glomerular ultrafiltrate may traverse the filtration barrier from blood into the urinary space. The barrier has three major components (Fig. 1.2):

1. **Capillary endothelial cells:** These are perforated by fenestrae up to 100 nm in diameter, close to which is an extensive network of filaments and microtubules. They have a negative surface charge due to the presence of podocalcyn, a polyanionic glycoprotein. The structure and function of endothelial cells will be discussed in more detail in Chapter 5.

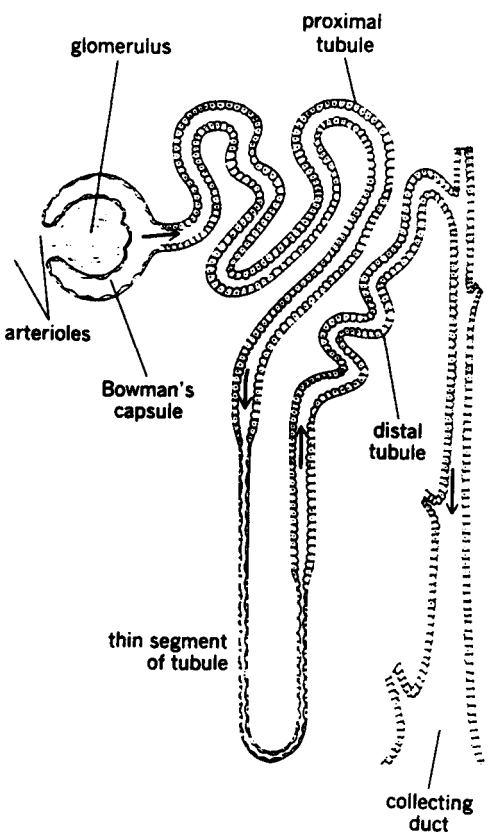

Fig. 1.1 The basic structure of a nephron.

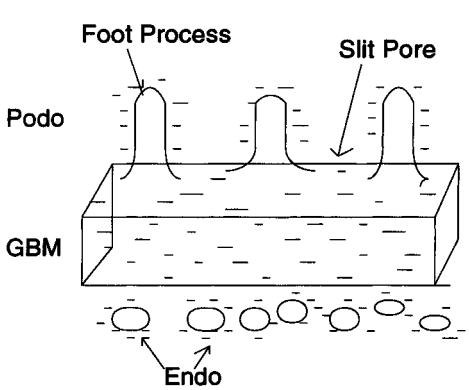

Fig. 1.2 The components of the glomerular filtration barrier. Podo: podocyte or epithelial cell. GBM: glomerular basement membrane. Endo: capillary endothelial cell.

2. **Glomerular basement membrane:** These also possess a fixed negative charge, the anionic sites consisting of glycosaminoglycans (GAGs) associated with procollagen-like molecules.
3. **Visceral epithelial cells:** These are also known as podocytes, and are the largest cells in the glomerulus. They possess long cytoplasmic trabeculae which divide into 'foot processes' and come into close contact with the glomerular basement membrane. The 'slit pores' are the gaps between adjacent foot processes. As with the other barrier components, the surface of the podocyte foot processes is negatively charged, due to the presence of sialic acid. Podocytes are responsible in part for the synthesis and maintenance of basement membrane components such as collagen, prostaglandins and GAG.

The glomerular mesangial cells, adjacent to the endothelium, are in fact specialised pericytes which possess smooth muscle cell and phagocytic properties. Their main function is to provide structural support for capillary loops, but contractile properties in response to vasoactive agents confer the ability to reduce glomerular filtration. The surrounding matrix is composed of sulphated GAG, fibronectin, and laminin. The cells of the proximal convoluted tubule contain membrane bound organelles (lysosomes) adjacent to the lumen, which are involved in endocytotic protein reabsorption. The subsequent tubular components are more fundamentally involved with electrolyte handling and urinary concentration and acidification, although distal tubular feedback in response to rate of urine flow and solute (particularly chloride) entry appears to then exert control over intrarenal (i.e. glomerular) haemodynamics, a process known as autoregulation.

Mechanisms of urinary protein excretion (Table 1.1)

Under physiological conditions, normal urine contains no more than one-millionth of the 12 600 g of protein filtered daily by the glomeruli. This reflects the efficiency of the glomerulus as a sieve, and the reabsorptive capacity of the tubular cells (Fig. 1.3). Perhaps no more than 60 % of urinary protein excretion is normally derived from the glomerular ultrafiltrate of plasma, the remainder produced by the kidney and the lower urinary tract. Glomerular and, to a lesser extent tubular protein handling are the most important determinants of abnormal patterns of protein excretion.

The initial glomerular ultrafiltrate is the net balance of the transcapillary hydraulic pressure and intravascular colloid osmotic pressure, respectively reflecting efferent arteriolar flow rate and pressure, and plasma protein concentration. As mentioned earlier, renal vascular flow is influenced by neural

Table 1.1. *Factors determining the constituents of excreted urinary protein*

Glomerular protein filtration
1. Renal plasma flow
2. Oncotic pressure
3. Protein size
4. Protein charge
5. Protein configuration
6. Glomerular basement membrane integrity and charge
Tubular protein handling
1. Tubular reabsorptive capacity
2. Competition from other proteins and solutes
3. Tubular secretion
4. Tubular catabolism
5. Tubulo-glomerular feedback
Renal tract protein excretion
1. Distal tubular secretion
Lower genito-urinary tract protein secretion and extravasation

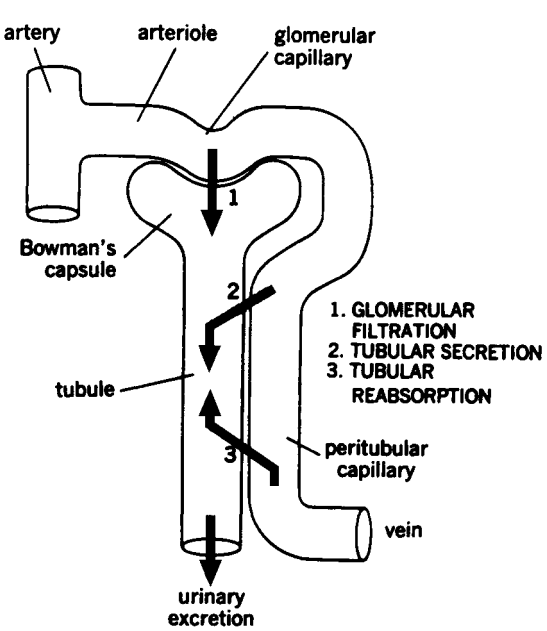

Fig. 1.3. The three basic components of normal urine production.

factors, tubulo-glomerular feedback, and also hormones and vasoactive substances (Table 1.2). Although renal blood flow usually reflects systemic blood flow and is a major determinant of glomerular filtration, it is attenuated by adrenergic neural activity. Furthermore, considerable variation in intrarenal haemodynamics is known to take place in response to local vasoconstrictive influences, such as angiotensin II and vasodilatory prostaglandins. Individual characteristics of plasma proteins (plasma concentration, size, charge, configuration and rigidity) and the integrity of the glomerular filtration barrier also determine the composition of the ultrafiltrate. Most circulating proteins have net negative charges, particularly those of the molecular weight of albumin or lower, and there is electrostatic repulsion between the protein molecules and the filtration barrier, since both are polyanionic. Molecules with neutral electrical charge such as IgG are less influenced by the polyanionic nature of the barrier, whereas molecules with a net positive charge more easily interact with the barrier[1]. Thus small uncharged molecules such as beta$_2$- microglobulin, retinol-binding protein and alpha$_1$-microglobulin (up to 30 kdaltons (kd)) pass freely into the urinary space in addition to water and electrolytes, whereas the passage of negatively charged molecules of intermediate size, such as albumin (67 kd), is normally impeded at the endothelial surface of the glomerular basement membrane.

Both diffusion and filtration (bulk flow) contribute to transglomerular protein passage. The selectivity index is based on the fractional excretion of two molecules of different size and charge, such as albumin and IgG. It can be used to confirm that the restrictive properties of the glomerular barrier remain intact. Factors such as non-enzymatic glycation (*vide infra*) may alter the charge of proteins and of the filtration barrier, thereby modifying this process[2]. Molecular heterogeneity of individual proteins may also alter glomerular handling in healthy subjects. For example, there is evidence that excessive binding of non-esterified fatty acids to albumin leads to distinctive changes in conformation, size, charge and ligand reactivity, with subsequent increased excretion and different chromatographic patterns of albuminuria[3]. This selectivity is lost in disease, when albumin with a low fatty acid content is excreted in greater amounts.

Thereafter, filtered proteins are normally reabsorbed by cells of the proximal convoluted tubule. This is achieved by pinocytosis, an energy-dependent process with high capacity but low affinity. As in the glomerulus, tubular cells selectively reabsorb proteins. The mechanism is not fully understood, although charge–charge interactions appear to influence the concentration of luminal proteins stored in endocytotic vesicles at the apex of tubular epithelial cells. Specific receptor-mediated uptake has not yet been demon-

Table 1.2. *Hormonal and vasoactive factors which modify renal blood flow*

Vasodilators	Vasoconstrictors
Prostaglandin E_1 and E_2	Angiotensin II
Serotonin (5HT)	Adrenaline
Acetylcholine	Insulin-like growth factor 1
Cyclic AMP	Epidermal growth factor
Endothelial-derived relaxing factor (nitric oxide)	Leukotrienes
Growth hormone	Endothelin
Atrial natriuretic peptide	Vasopressin
Bradykinin	
Insulin	
Insulin-like growth factor I (pharmacological effect)	
Glucagon	

strated, but it does appear that proteins may compete for uptake, with increased filtration of either low or high molecular weight proteins leading to increased urinary excretion of other proteins[4,5]. Tubular protein uptake may also be modified by urinary flow rates, and impeded by solute components such as glucose, amino acids, ketone bodies and toxic proteins such as immunoglobulin light chains. Following reabsorption, partial intracellular hydrolysis to amino acids, and then secretion into the circulation takes place. Under certain conditions, transtubular transport of intact protein may occur. Fractional reabsorption of low molecular weight proteins in healthy individuals has been estimated at 99.97 %, whereas that of albumin varies between 92 and 99 %.

Direct tubular secretion of proteins also contributes to urinary protein excretion. The Tamm Horsfall mucoprotein is a large (23 000 kd) acidic glycoprotein derived from the epithelial surface membranes of the thick ascending loop of Henle and the early distal convoluted tubule. It is the major constituent of urinary casts, and has recently been shown to be identical in sequence to uromodulin, suggesting a common role in inactivation of lymphokines such as interleukin (IL-1) and tumour necrosis factor.

Secretory IgA normally accounts for <3 % of urinary protein. It is secreted from tubular epithelial cells to help maintain a sterile urine. Urokinase is one of several urinary enzymes of intermediate size (31 and 55 kd) which appear in normal urine. It probably acts as an antifibrinolytic agent and may help in cast removal. Tubular secretion of enzymes such as n-acetyl-glucosaminosidase (NAG) may have functional importance, and excretion of high concentrations may reveal tubular damage. Lesser amounts

of urinary proteins and glycoproteins are also derived from seminal, prostatic, urethral and vaginal secretion, but ejaculation does not appear to influence the albumin excretion rate[6].

Structure and function of albumin and other plasma proteins excreted in normal urine (Table 1.3)

Albumin is the most abundant plasma protein (usual concentration 36–50 g/l). It is derived from the liver, and synthetic rates are highly responsive to any change in requirements consequent upon loss of circulating albumin. It has a relatively long half-life of 2–3 weeks. Its main functions are to maintain colloid osmotic pressure within the intravascular compartment, and as a transporter of ions, water insoluble substances such as lipids and non-esterified fatty acids, hormones and drugs. Catabolism of albumin takes place predominantly in the liver and kidney.

Retinol-binding protein (RBP) is an α-globulin, which is synthesised in the liver, and circulates in the blood bound to prealbumin. The size of the circulating complex prevents filtration through the glomerulus. The affinity for prealbumin lessens following delivery of retinol to the target epithelial tissues, when RBP then undergoes complete glomerular filtration and catabolism in the proximal renal tubules. In normal subjects serum levels of RBP are over one thousand times higher than in urine[7].

β_2-microglobulin is a cationic low molecular weight protein, which is thought to be produced by normal white blood cells, and may be related to the human leucocyte antigen (HLA) complex. It is freely filtered by the glomerulus, but is not ideal as a marker of tubular function, as it is unstable in urine, particularly at low pH.

α_1-microglobulin is a glycoprotein synthesised in the liver, which appears to be stable in acidic urine. It may bind to IgA and influence immune function[8]. IgG is one of the group of immunoglobulins synthesised by plasma cells and which function as antibodies. They are synthesised from two heavy and two light polypeptide chains. They form particularly in response to soluble antigens such as bacterial toxins. Components of IgG such as kappa or gamma light chains may also circulate and are freely filtered by the glomerulus. Some 96 % of IgG is either neutral or cationic in charge, which is in marked contrast to IgG4, the anionic subclass which is preferentially excreted in normoalbuminuric subjects[9].

Transferrin is the major iron-binding protein present in plasma. It transports iron from the sites of absorption and red cell breakdown, to the developing red cells in bone marrow. The binding sites of transferrin are normally

Table 1.3. *Size, charge, and renal excretion of plasma proteins in normal subjects*

Name	Molecular weight (Daltons)	Charge	Normal 24 h excretion (μg/min; range)
Albumin	67 000	—	0.100–15.000
Retinol-binding protein	21 000	0	0.016–0.173
β_2-microglobulin	11 815	0	0.021–0.257
IgG	160 000	0	1.340–1.700
Transferrin	90 000	0	0.090–0.326
α_1-microglobulin	30 000	0	0.902–6.250
α_1-acid glycoprotein	44 000	0	0.125–0.469
$\alpha_1$antitrypsin	45 000	0	0.130–0.391
Caeruloplasmin	160 000	—	0.032–0.042
Haptoglobin	85 000	—	0.000–0.290
G_c-globulin	50 000	0	0.011–0.033
Haemopexin	80 000	—	0.099–0.199
β_2-glycoprotein	40 000	0	0.150–0.338

Charge: 0 neutral;—anionic

about 30 % saturated. It has a slightly larger molecular weight than albumin, but is of greater molecular charge, favouring tubular reabsorption over albumin.

Physiological determinants of proteinuria

Although a large number of plasma protein constituents of normal urine have been identified (Table 1.3), the majority of proteins are not derived from blood but, like Tamm Horsfall protein, are secreted from the kidney or from the lower genito-urinary tract. Urinary excretion of albumin and lower molecular weight proteins is enhanced by any factors which increase the load filtered by the glomerulus, either by saturating tubular reabsorptive capacity, or simply as a result of increased tubular volume and flow rate. Thus, increased proteinuria in normal subjects would result from any situation of increased renal blood flow and glomerular filtration rates and/or increased intraglomerular pressure and permeability. This is most clearly seen during extracellular volume expansion with saline, but even simple oral water loading appears to increase acutely the urinary excretion of albumin [10–12]. The effect of water loading on excretion of retinol-binding protein and other lower molecular weight proteins is less marked.

Urinary albumin excretion also increases during the assumption and maintenance of an upright posture, regardless of the time, although particularly by day, suggesting additional diurnal variation. The actual magnitude of postural variation varies considerably between individuals, and within-individual day-to-day variation of up to 80 % is recognised. The mechanism is presumed to be glomerular, the consequence of an increased filtration fraction due to respective increases and reductions in systemic blood pressure and renal blood flow. This is supported by the observation that normally renal blood flow will increase and systemic and renal vascular resistance will fall whilst recumbent. This is not the case if there is autonomic dysfunction. Upright posture does not appear to increase excretion of retinol-binding protein, strengthening the view that tubular proteinuria is not normally influenced by postural changes in blood flow and vascular resistance. Exercise-induced increase in albumin excretion is well recognised[13], and is the likely consequence of increases in renal blood flow, systemic and intraglomerular pressure and filtration fraction.

Although it has been argued that albuminuria after exercise predominantly reflects a failure of proximal tubular reabsorption, excretion of lower molecular weight proteins may not necessarily increase during exercise[13]. This suggests either an alternative mechanism for the increased albuminuria, or could be compatible with the concept of selective tubular reabsorption of smaller molecular weight proteins, in preference to albumin. Oral glucose loading in healthy individuals also acutely increases albuminuria although not low molecular weight proteinuria[14]. Tubular protein–protein interaction is again the likeliest explanation for this phenomenon, although acute increases in renal blood flow may also be implicated, in part as a response to physiological hyperinsulinaemia. By contrast, acute and chronic dietary protein loads appear to increase both albuminuria and low molecular weight proteinuria, primarily as a result of the increased glomerular filtration and urine flow rate which is a homeostatic response to the increased nitrogenous load[10,15].

Pregnancy is another physiological state of increased urinary protein excretion[16,17]. Urinary albumin excretion appears to increase some threefold in the third trimester, and similar changes in other proteins, including retinol binding protein and transferrin, have been recorded. The mechanism is likely to be complex, and not simply the result of increased renal blood flow and intraglomerular pressure, but may also be due to increased protein synthesis, selective filtration and tubular reabsorption, and altered capillary permeability, reflecting physiological changes in neuro-endocrine balance.

References

1. Christensen EI, Rennke HG, Carone FA. Renal tubular uptake of protein: effect of molecular charge. *Am J Physiol* 1983; **244**:F436–F441.
2. Cavallo-Perin P, Chiambretti A, Calefato V, Tomalino M, Cecchini G, Gruden G, Pagano G. Urinary excretion of glycated albumin in insulin-dependent diabetic patients with micro- and macroalbuminuria. *Clin Nephrol* 1992; **38**:9–13.
3. Hayashi Y, Morikawa A, Makino M. Heterogeneity of urinary albumin from diabetic patients. *Clin Chim Acta* 1990; **190**:93–104.
4. Bernard A, Viau C, Ouled A, Lauwerys R. Competition between low- and high- molecular weight proteins for tubular uptake. *Nephron* 1987; **45**:115–118.
5. Nguyen-Simonnet H, Vincent C, Revillard JP. Competition between albumin and beta-2-microglobulin for renal tubular uptake: brush border and/or lysosomes? *Nephron* 1988; **48**:159–160.
6. Hirsch IB, Farkas-Hirsch R, Herbst JS, Skyler JS. The effect of ejaculation on albumin excretion rate. *J Diabet Compl* 1992; **6**:163–165.
7. Tomlinson PA, Dalton RN, Turner C, Chantler C. Measurement of $ß_2$-microglobulin, retinol-binding protein, $alpha_1$-microglobulin and urine protein I in healthy children using enzyme-linked immunosorbent assay. *Clin Chim Acta* 1990; **192**:99–106.
8. Yu H, Yanagisawa Y, Forbes MA, Cooper EH, Crockson RA, MacLennan ICM. Alpha-1-microglobulin: an indicator protein for renal tubular function. *J Clin Pathol* 1983; **36**:253–259.
9. Pietravalle P, Morano S, Cristina G, Grazia de Rossi M, Mariani G, Cotroneo P, *et al.* Charge selectivity of proteinuria in type 1 diabetes explored by Ig subclass clearance. *Diabetes* 1991; **40**:1685–1690.
10. Amore A, Coppo R, Rocattello D, Martina G, Rollino C, Basolo B *et al.* Single kidney function: effect of acute protein and water loading on micro-albuminuria. *Am J Med* 1988; **84**:711–715.
11. First MR, Sloan DE, Pesce AJ, Pollak VE. Albumin excretion by the kidney: The effect of volume expansion. *J Lab Clin Med* 1977; **89**:25–29.
12. Viberti GC, Mogensen CE, Keen H, Jacobsen FK, Jarrett RJ, Christensen CK. Urinary excretion of albumin in normal man: the effect of water loading. *Scand J Clin Lab Invest* 1982; **42**:147–151.
13. Watts GF, Williams I, Morris RW, Mandalia S, Shaw KM, Polak A. An acceptable exercise to study microalbuminuria in type 1 diabetes. *Diabet Med* 1989; **6**:787–792.
14. Hegedus L, Christiansen NJ, Mogensen CE, Gundersen HJG. Oral glucose increases urinary albumin excretion in normal subjects but not in insulin-dependent diabetics. *Scand J Clin Lab Invest* 1980; **40**:479–480.
15. Shestakova MV, Mukhin NA, Dedov II, Titov VN, Warshavsky VA. Protein-loading test, urinary albumin excretion and renal morphology in diagnosis of subclinical diabetic nephropathy. *J Intern Med* 1992; **231**:213–217.
16. Lopez-Espinoza I, Dhar H, Humphreys S, Redman WG. Urinary albumin excretion in pregnancy. *Br J Obstet Gynaecol* 1986; **93:**176–181.
17. Wright A, Steele P, Bennett JR, Watts G, Polak A. The urinary excretion of albumin in normal pregnancy. *Br J Obstet Gynaecol* 1987; **94**:408–412.