Tubular Reabsorption and Secretion 7.4

Learning Objectives

- Calculate the filtered load from the sieving coefficient, plasma concentration, and GFR
- From the filtered load and rate of excretion, calculate the rate of secretion or reabsorption
- From a renal titration curve, identify T_m, renal threshold, and splay
- List the causes of splay in renal titration curves
- From renal titration curves, identify substances that are reabsorbed or those that are secreted
- Explain why endogenous creatinine clearance is a good estimate of the GFR
- Describe the mechanism of glucose reabsorption in the proximal tubule
- Explain why glucose appears in the urine of diabetic persons
- Describe the mechanism of amino acid and phosphate reabsorption in the proximal tubule
- Explain how the double ratio of $(TF/P)_x/(TF/P)_{inulin}$ illuminates the site of reabsorption
- Estimate the fraction of water and electrolytes that are absorbed in the proximal tubule
- Describe the mechanism of protein reabsorption

THE *FILTERED LOAD* OF WATER AND VALUABLE NUTRIENTS IS ENORMOUS

For many small molecular weight solutes, such as amino acids, glucose, and electrolytes, the sieving coefficient, Θ, at the glomerulus is about 1.0—the concentration of the materials in the ultrafiltrate is the same as in plasma. The rate of filtration of any plasma material can be calculated as

$$[7.4.1] \qquad \text{Filtered load}_x = \text{GFR}\ \Theta_x\ P_x$$

where GFR is the glomerular filtration rate, normally expressed in mL min^{-1}, Θ_x is the sieving coefficient for substance x, and P_x is the plasma concentration of substance x. The normal GFR is about 120 mL min^{-1} or about 170 L day^{-1}. For a 70-kg adult male, the total body water is only 42 L. Thus every day the kidneys filter an equivalent of about four times the entire body water. Since the total urine output is only 1−2 L day^{-1}, most of the filtered load of water must be returned to plasma and not excreted in the urine. The kidneys reabsorb nearly the entire filtered load of water.

THE RENAL TITRATION CURVE OF INULIN IS LINEAR

We can find insights into the overall handling of materials by the kidneys by examining the rate of excretion as a function of the plasma concentration. In this process, we vary plasma concentration, typically by infusing increasing amounts of the material being studied, and we measure the urine flow rate (Q_u), urinary concentration (U_x), and plasma concentration (P_x). We then calculate the rate of excretion as Q_uU_x and plot this against P_x. The result of such an experiment using inulin as a test substance is shown in Figure 7.4.1. We can analyze these curves using the equations that we presented in Chapter 7.3. During the steady state, the overall renal handling of inulin is described by the conservation relation, assuming that inulin is neither destroyed nor produced metabolically.

$$[7.4.2] \qquad \text{Input} = \text{output}$$

Considering the origin of fluxes into and out of the tubule, we write

$$[7.4.3] \qquad \text{Rate of filtration} + \text{rate of secretion} = \text{rate of excretion} + \text{rate of reabsorption}$$

which is identical to Eqn (7.3.3). In the case of inulin, the rate of excretion is proportional to the plasma [inulin] and is linearly related to the rate of filtration. This linear relationship must hold true if inulin is freely filtered but is neither reabsorbed nor secreted, but the linear relationship by itself is not proof that inulin is handled this way by the kidney. Other experiments have shown that inulin is indeed neither secreted nor reabsorbed. If a single nephron is blocked just below the glomerulus and inulin is perfused into the nephron just below the block, the inulin is quantitatively recovered in the distal loop of the same nephron, indicating that none is reabsorbed. If inulin is also perfused systemically, the recovery of inulin is unchanged. This indicates that inulin is not secreted. The way inulin is handled by the kidney allows us to use its clearance to estimate the GFR accurately.

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EXAMPLE 7.4.1 Calculate the Daily Filtered Load of Glucose and Na^+

The normal plasma glucose concentration is about 80 mg dL^{-1}. Since $\Theta_{glucose} = 1.0$, we can calculate the daily filtered load as

GFR $\Theta_{glucose} P_{glucose}$ = 170 L day^{-1} × 1.0 × 80 mg dL^{-1} × 10 dL L^{-1} = **136 g glucose day^{-1}**

The normal plasma Na^+ is about 142 mmol L^{-1}, and it is readily filtered because it is small: $\Theta_{Na} = 1.0$.

The daily filtered load is

GFR $\Theta_{Na} P_{Na}$ = 170 L day^{-1} × 1.0 × 142 mmol L^{-1} = 24,140 mmol day^{-1}

This is 24.14 mol × 22.99 g mol^{-1} = **555 g day^{-1}**.

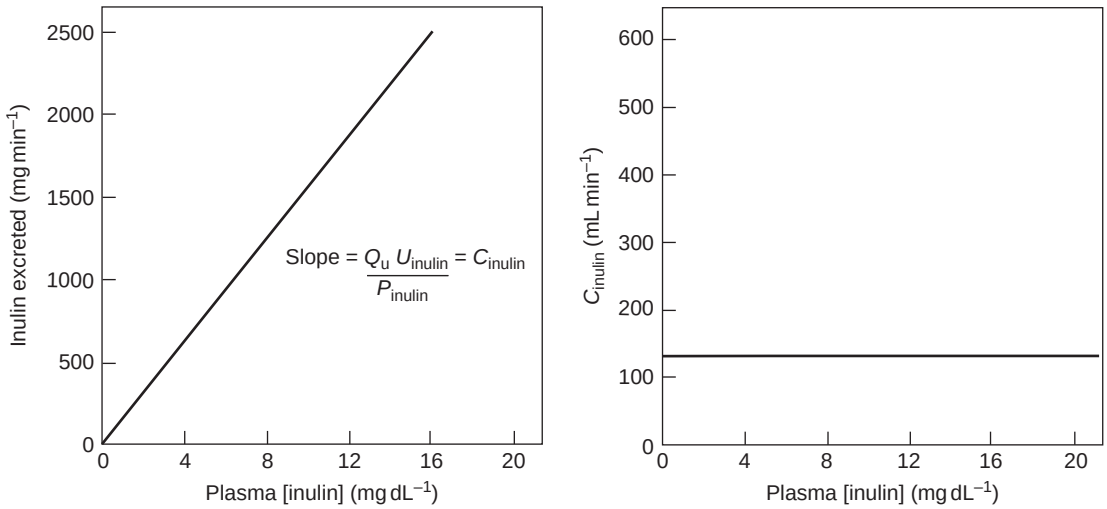

FIGURE 7.4.1 Renal titration of the kidneys with inulin. The rate of excretion of inulin is linearly related to its plasma concentration (left). The ratio of the rate of excretion ($Q_u U_{inulin}$) to the plasma [inulin] (P_{inulin}) is the clearance of inulin in units of mL min^{-1}, which is the slope of the line of rate of excretion versus P_{inulin}. A typical value for an adult human male is about 120 mL min^{-1}. In the case of inulin, the clearance is independent of its plasma concentration (right).

In Example 7.4.1, we calculated that the daily filtered load of glucose is 136 g. Normally there is zero glucose in the urine. Thus all of this filtered glucose is reabsorbed by the kidney. The filtered load of Na^+ is calculated to be about 555 g Na^+ day^{-1}. Typically the urinary excretion of Na^+ is about 4 g day^{-1}. Thus the kidneys reabsorb some 551 g of Na^+ from the ultrafiltrate every day. These numbers show that the filtered load of many substances is enormous and that a major job of the kidney is reabsorbing all of these necessary materials from the ultrafiltrate.

THE RENAL TITRATION OF GLUCOSE SHOWS REABSORPTION AND SATURATION KINETICS

We can perform a renal titration of glucose by infusing glucose at constant rates to establish different steady-state plasma [glucose]. The rate of glucose filtration will vary linearly with the $P_{glucose}$ according to Eqn (7.4.1). The rate of excretion is $Q_u \times U_{glucose}$, meaning the urinary flow times the urinary [glucose]. We have

$$\begin{aligned}&\text{Rate of filtration} = \text{GFR}\Theta_{glucose}P_{glucose}\\&\text{Rate of secretion} = 0\\&\text{Rate of excretion} = Q_u U_{glucose}\\&\text{Rate of reabsorption} = \text{GFR}\Theta_{glucose}\,P_{glucose} - Q_u U_{glucose}\end{aligned} \quad [7.4.4]$$

The amount filtered per unit time is a linear function of the plasma glucose concentration, as described in Eqn (7.4.4), with slope = GFR × $\Theta_{glucose}$ when plotted against $P_{glucose}$ where the sieving coefficient for glucose = 1. The amount excreted per unit time is experimentally determined. The experimental observation is that urinary excretion of glucose is zero until plasma [glucose] concentrations exceed a **renal threshold**. This is the plasma glucose concentration at which glucose first appears in the urine. After a short nonlinear portion called **splay**, the amount of glucose excreted per unit time increases linearly with further increases in plasma glucose. From these two quantities, the rate of filtration and the rate of excretion, we can calculate the rate of glucose reabsorbed. The results are shown in Figure 7.4.2.

The titration curve for glucose (see Figure 7.4.2) shows that the rate of reabsorption of glucose increases

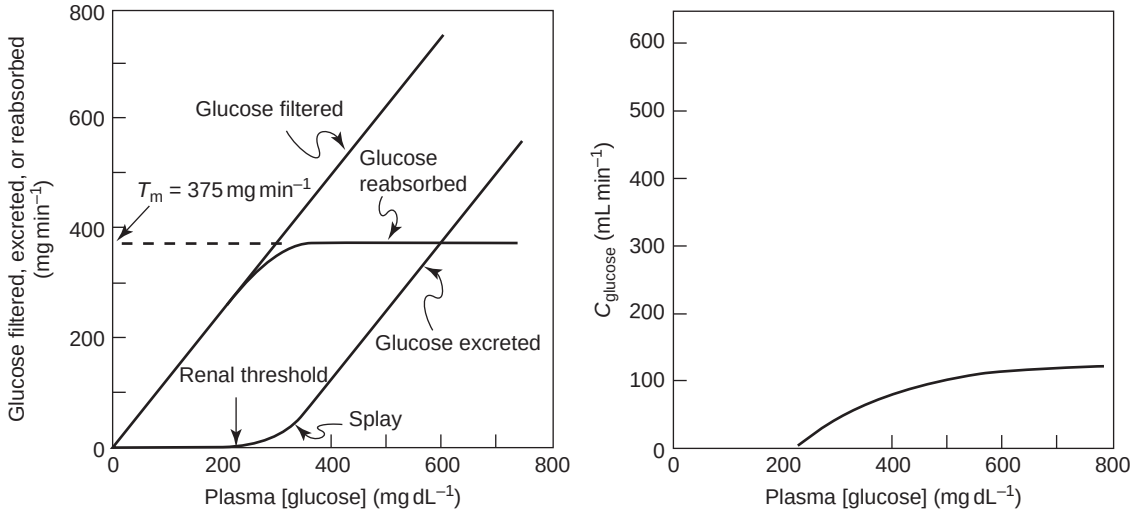

FIGURE 7.4.2 Titration of the renal tubules of man with plasma glucose. Plasma glucose concentration was gradually increased by infusing glucose. The rate of glucose excretion was measured simultaneously with the clearance of inulin and plasma glucose concentration. The amount filtered per unit time was calculated as $C_{inulin} \times P_{glucose}$, and the amount reabsorbed per unit time was calculated as the amount filtered per unit time minus the amount excreted per unit time. The amount reabsorbed shows saturation kinetics with a well-defined transport maximum, $T_{m\ glucose}$. *Redrawn from R.F. Pitts,* Physiology of the Kidney and Body Fluids, *Year Book Medical Publishers, Chicago, IL, 1974.*

with the filtered load until a maximum rate is reached and then reabsorption does not increase further. The transport mechanism displays **saturation kinetics** and the kidney has a distinct transport maximum or T_m. The transport maximum for glucose, $T_{m\ glucose}$, is about 375 mg min^{-1}. With a GFR of 120 mL min^{-1}, the filtered load just equals the T_m when the plasma glucose concentration is about 312 mg dL^{-1}. However, glucose begins appearing in the urine before this value of plasma glucose concentration is reached.

SATURATION KINETICS AND NEPHRON HETEROGENEITY CAUSE SPLAY

Gradual saturation is typical of carriers that bind transported substrate. The transport rate initially increases nearly linearly with the concentration of transported material and then gradually tapers off. The curve of rate versus concentration of transported material does not show sharp breaks (see Chapter 2.5). The second explanation for splay is the heterogeneity of nephrons. Nephrons are not uniformly long and their glomeruli are not all the same size. Their relative rate of ultrafiltration and reabsorption can vary. This is **morphologic heterogeneity**. Thus not all nephrons have the same transport maximum, and the overall observed T_m is a weighted average of all the individual nephron T_m.

HIGH PLASMA GLUCOSE IN *DIABETES MELLITUS* CAUSES GLUCOSE EXCRETION

The normal plasma glucose concentration is about 80–120 mg dL^{-1}, and therefore the normal filtered load is about 100–150 mg min^{-1}. This is far below $T_{m\ glucose}$. Usually the urine contains no glucose. Its clearance, defined as

$$[7.4.5] \qquad C_G = \frac{Q_u U_G}{P_G}$$

is normally zero. Here C_G is the clearance of glucose, Q_u is the urine flow rate, in mL min^{-1}; U_G is the urine concentration of glucose, which is generally zero; and P_G is the plasma [glucose]. C_G is zero at low plasma [glucose] but rises with higher plasma [glucose], as shown in Figure 7.4.2, to approach the clearance of inulin at very high plasma [glucose]. The clearance at any P_G is the slope of the chord in Figure 7.4.2 connecting the origin to the curve for the rate of glucose excretion.

Because plasma [glucose] is usually regulated at levels below the renal threshold, **the kidneys normally do not participate in the homeostatic regulation of plasma [glucose]**. Instead, plasma glucose homeostasis is controlled by hormones including insulin, glucagon, epinephrine, and glucocorticoids. Insulin is secreted by the β cells of the islets of Langerhans in the pancreas (see Chapter 9.4) and stimulates glucose uptake by peripheral tissues. If insulin secretion is inadequate, or if the target tissues become insensitive to insulin, then plasma [glucose] rises. If uncorrected, this hyperglycemia can exceed the renal threshold and glucose appears in the urine. This condition is called **glucosuria** (or glycosuria) and the disease is called **diabetes mellitus**. The excreted glucose exerts an osmotic effect that markedly increases urine flow, Q_u. This explains the name of the disease: "diabetes" means "to siphon off" and "mellitus" means "sweet." This describes the sweet taste of the copious and dilute urine produced by persons with uncontrolled diabetes mellitus. The uncontrolled disease is characterized by frequent drinking **(polydipsia)** to replenish fluids lost by frequent urination **(polyuria)** and weight loss due to loss of calories in the form of glucose.

THE KIDNEYS HELP REGULATE PLASMA PHOSPHATE

The renal titration for phosphate PO_4^- is shown in Figure 7.4.3. At physiological pH, plasma phosphate exists as HPO_4^{-2} and $H_2PO_4^{-1}$, and so plasma [phosphate] is expressed as their sum, in mM, or in terms of mg phosphorus dL^{-1}. Normal plasma [phosphate] is about 0.9–1.5 mM. The filtered load shown in Figure 7.4.3 is a linear function of the plasma [phosphate], with the slope of the line being the GFR. The equation for the line is given by Eqn (7.4.1). The renal threshold for phosphate occurs at about 1 mM. Increasing plasma [phosphate] above the renal threshold increases the amount of phosphate that is excreted. As with glucose, the relationship between excreted phosphate and plasma [phosphate] above the renal threshold is linear, with a slope equal to the GFR. The amount of phosphate absorbed shows a distinct $T_{m\ phosphate}$ of about 0.125 mmol min^{-1}. Thus the overall handling of phosphate by the kidneys is similar in form to that of glucose. However, the renal threshold for phosphate is close to the physiologically normal plasma [phosphate], so that the urine nearly always contains some amount of phosphate. Because of this, **the kidneys participate in the normal regulation of plasma [phosphate]**. If plasma [phosphate] increases, the filtered load increases. Because the filtered load already slightly exceeds the renal threshold, more filtered load means that the $T_{m\ phosphate}$ is further exceeded and more phosphate is excreted in the urine. Rises in plasma [phosphate] increase phosphate excretion so that plasma [phosphate] returns towards normal.

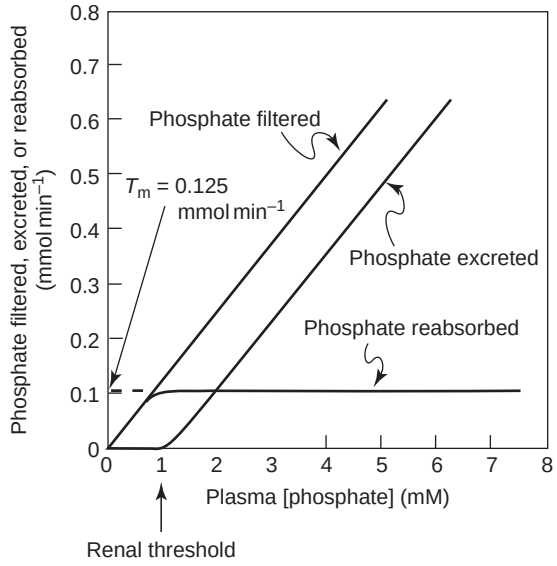

FIGURE 7.4.3 Renal titration of plasma phosphate. The filtered load increases linearly with the plasma [phosphate]. Above about 1 mM, increases in excreted phosphate are linear with the increases in plasma phosphate, with a slope equal to the GFR. The rate of phosphate reabsorption can be calculated from the difference between the filtered load and the rate of phosphate excretion. The resulting curve shows saturation kinetics, with a well-defined transport maximum, $T_{m\ phosphate}$ that is about 0.125 mmol min^{-1}. Since $T_{m\ phosphate}$ is nearly the same as the normal filtered load, the kidney acts like a spillway for phosphate: it keeps phosphate at the level of the spillway and any excess drains off into the urine. *Redrawn from R.F. Pitts,* Physiology of the Kidney and Body Fluids, *Year Book Medical Publishers, Chicago, IL, 1974.*

RENAL TITRATION CURVE OF PAH SHOWS SECRETION

The renal titration of *para*-amino hippuric acid (PAH) is shown in Figure 7.4.4. This titration curve differs from the renal titration curves of glucose or phosphate

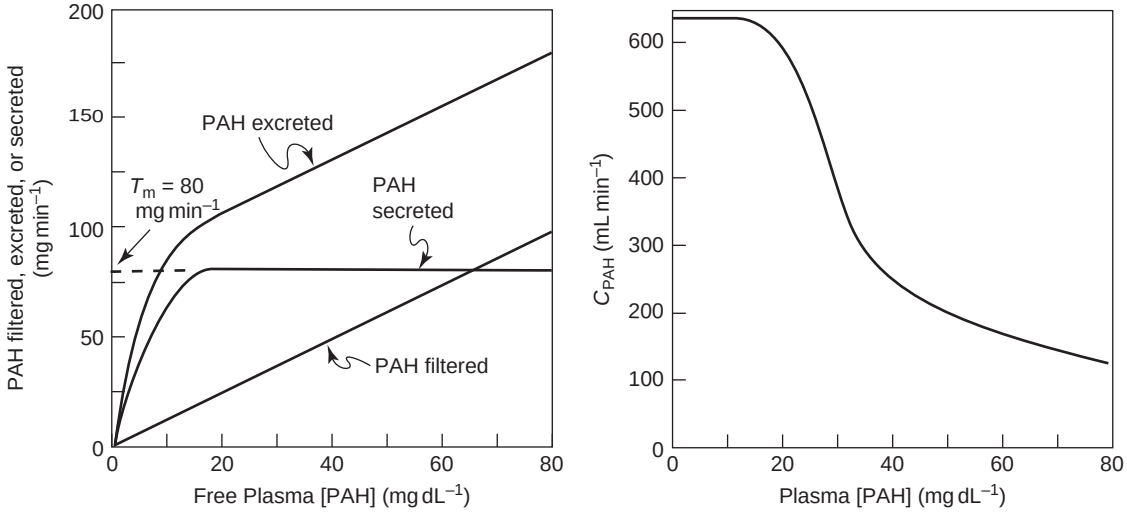

FIGURE 7.4.4 Renal titration of PAH. Increasing the plasma [PAH] increases the rate of PAH excretion. At low plasma [PAH], $T_{m\ PAH}$ is not exceeded and all blood perfusing the secreting portions of the nephrons is cleared of PAH. In this region of plasma [PAH], the clearance is equal to the effective renal plasma flow, ERPF. At higher plasma [PAH] the clearance falls because the transport mechanism is saturated and some plasma PAH escapes in the renal venous blood.

because the rate of excretion of PAH exceeds its rate of filtration. The rate of filtration is calculated as $GFR \times P_{PAH}$ ($\Theta_{PAH} = 1.0$). The rate of excretion can exceed the rate of filtration only if the material is secreted. The rate of secretion is calculated by using Eqn (7.4.3):

$$\text{Rate of secretion} = \text{rate of excretion} - \text{rate of filtration} \quad [7.4.6]$$

The principle of secretion was first established for injected dyes whose concentration could be easily measured and whose route in the kidney could be traced microscopically. The rate of secretion of PAH obeys saturation kinetics with a well-defined $T_{m\ PAH}$.

The clearance of PAH is constant and high at low plasma [PAH], below the $T_{m\ PAH}$. Under these circumstances the blood that perfuses the secreting portions of the nephron is nearly completely cleared of PAH. Under these conditions, the clearance of PAH approximates the effective renal plasma flow, ERPF. At high P_{PAH} that exceeds the renal threshold, the clearance no longer approximates the ERPF. The clearance in Figure 7.4.4 is the slope of the chord connecting the origin to the curve for rate of PAH excretion.

THE MEANING OF THE CLEARANCE DEPENDS ON THE RENAL HANDLING

Figures 7.4.1, 7.4.2, and 7.4.4 show the dependence of the clearance on the plasma concentration of three different solutes: inulin, glucose, and PAH. The clearance for inulin is independent of plasma [inulin]. The clearance of glucose is zero at low plasma [glucose] and climbs asymptotically toward the inulin clearance when plasma [glucose] increasingly exceeds its T_m. The clearance of PAH is highest at lowest plasma [PAH] and it asymptotically approaches the inulin clearance as plasma [PAH] increasingly exceeds its secretory T_m. Figure 7.4.5 shows all of these curves together plus an additional curve for the clearance of creatinine. These curves illustrate that the clearance of a solute depends on its concentration and on how the kidney handles it. For inulin, the clearance is equal to the GFR; for glucose, the clearance is normally zero, meaning that it is entirely reabsorbed; for PAH, the clearance at plasma [PAH] below $T_{m\ PAH}$ is a measure of ERPF.

ENDOGENOUS CREATININE CLEARANCE APPROXIMATES THE GFR

Creatinine is a by-product of muscle metabolism in which creatine in the muscle is converted nonenzymatically to creatinine. Because the total body content of creatine is fairly constant, there is a continual production of creatinine and a continual excretion of it in the urine. The typical 70-kg adult man produces about 2 g of creatinine per day. As evident from Figure 7.4.5, creatinine is slightly secreted by the kidneys so that at low plasma [creatinine] the clearance of creatinine is about 5–10% greater than the inulin clearance. However, creatinine is already present in the blood at steady-state

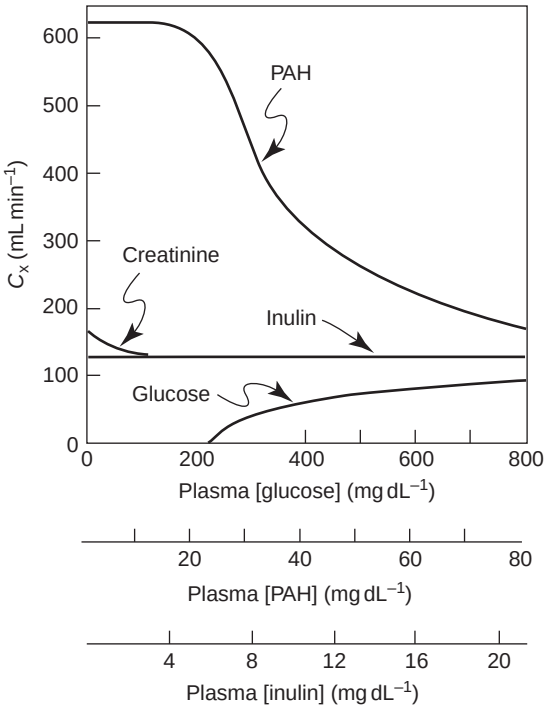

FIGURE 7.4.5 Comparison of the clearances of inulin, glucose, PAH, and creatinine as a function of their plasma concentrations. The clearance of inulin is independent of its concentration and is interpreted as being equal to the GFR because inulin is freely filtered and not reabsorbed or secreted. The clearance of glucose is zero at physiological plasma [glucose] because glucose is avidly reabsorbed by the renal tubules. The clearance of PAH is high at low plasma [PAH] because the kidneys avidly secrete it into the tubular fluid. The clearance of creatinine is about 10% higher than the inulin clearance at physiological plasma [creatine]. The clearance of endogenous creatinine can be used to approximate the GFR.

levels, so the **endogenous creatinine clearance** can be used without the necessity of having to infuse inulin. All that is necessary is a timed urine sample and a plasma sample. Measuring the [creatinine] in both the urine and plasma sample allows the calculation of the endogenous creatinine clearance that provides an approximate measure of the GFR.

PLASMA CREATININE CONCENTRATION ALONE INDICATES THE GFR

At steady state, the rate of creatinine production by the body will be equal to its rate of excretion. The rate of excretion is $Q_u U_{creatinine}$. The major source of the excreted creatinine is from ultrafiltration, with a small fraction from secretion. Thus we can write

$$Q_u U_{creatinine} = GFR P_{creatinine} + T_s \quad [7.4.7]$$

where T_s, the rate of secretion of creatinine, is generally small compared to the rate of filtration. The rate of excretion is relatively constant. Dividing both sides of Eqn (7.4.7) by GFR, we obtain

$$\frac{Q_u U_{creatinine} - T_s}{GFR} = P_{creatinine} \quad [7.4.8]$$

Since T_s is generally small compared to the other term in the numerator, there is an approximate inverse relationship between the GFR and plasma [creatinine]. The normal production of creatinine is about 2 g day^{-1} or about 1.39 mg min^{-1}, which is equal to $Q_u U_{creatine}$ in the numerator. Because T_s is small, the total rate of filtration, the amount in the numerator in Eqn (7.4.8), is about 1.3 mg min^{-1}. If the GFR is 120 mL min^{-1}, the plasma [creatinine] should be about 1.3 mg min^{-1}/120 mL min^{-1} $\approx$ 1.1 mg dL^{-1}. Average values of plasma [creatinine] range from 0.5 to 1.5 mg dL^{-1}, with an average of 1.2 mg dL^{-1}.

Clinicians have developed two widely used equations that estimate the GFR from serum creatinine measurements. These are the Modification of Diet in Renal Disease (MDRD) Study equation and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. These use measurements of serum creatinine from the isotope dilution mass spectrometer method. The MDRD equation is

$$\text{GFR}(\text{mL min}^{-1}/1.73\,\text{m}^2) = 175 \times [\text{Cr}]^{-1.154} \times \text{Age}^{-0.203} \times \text{K} \quad [7.4.9]$$

the constant, K, is adjusted for different groups. It is 0.742 if the patient is a female, and 1.212 if the patient is African American. The CKD−EPI equation is actually a set of eight equations with slightly different constants depending on race, gender, and values of the measured serum creatinine. These all take the form

$$\text{GFR} = \text{K} \times \left(\frac{[\text{Cr}]}{\alpha}\right)^{-\beta} \times 0.993^{\text{Age}} \quad [7.4.10]$$

where the constants K, α, and β differ depending on the range of the creatine measurement or if the patient is male or female or Caucasian or black. These equations have the advantage of avoiding errors in the 24-hour urine collection necessary for the creatinine clearance measurement, but cannot be used for persons with abnormal creatinine production. Abnormal creatinine production may occur in persons with extreme body size or muscle mass, such as amputees, paraplegics, morbidly obese persons or persons eating vegetarian diets or taking creatine supplements.

$(TF/P)_{INULIN}$ MARKS WATER REABSORPTION

The GFR is about 120 mL min^{-1}, whereas urine flow is about 1 mL min^{-1}. Thus most of the ultrafiltrate fluid is reabsorbed. Where in the nephron is it absorbed? We can use the ratio of concentrations TF/P for inulin to get an answer. Because the sieving coefficient is 1.0 for inulin, its concentration in Bowman's space is the same as it is in plasma: the ratio $(TF/P)_{inulin} = 1.0$ at this point in the nephron. Once the inulin is filtered, it is neither reabsorbed nor excreted, but water reabsorption from the tubular fluid will concentrate the remaining inulin and $(TF/P)_{inulin}$ will increase. Let V_{TF} be the volume of the tubular fluid that remains in the tubule and passes through a particular cross-section of the nephron, per second, and let V_R be the volume of fluid that has been reabsorbed by the nephron from the glomerulus up to that point (see Figure 7.4.6). The total volume of these two is just the volume of ultrafiltrate formed, V_T, per second:

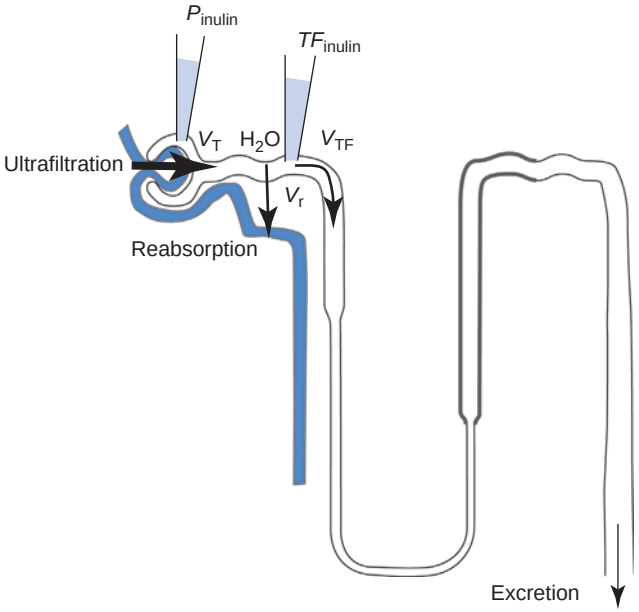

FIGURE 7.4.6 Simplified nephron showing the sampling of tubular fluid at Bowman's capsule and further down the nephron. The volume of filtrate is V_T, which is equal to the volume of fluid remaining in the tubule at the place of sampling, V_{TF}, plus the volume of fluid that is reabsorbed, V_R. The concentration of inulin in the ultrafiltrate is equal to its plasma concentration, P_{inulin}, and its concentration at the point of sampling of tubular fluid is designated as TF_{inulin}.

$$V_{TF} + V_R = V_T \quad [7.4.11]$$

At steady state, the total amount of inulin that remains in the tubule and passes by any point in the nephron per second must be the same everywhere or else there will be a buildup or depletion of the amount of inulin:

$$V_{TF}TF_{inulin} = V_T P_{inulin} \quad [7.4.12]$$

We use the plasma [inulin], P_{inulin}, on the right-hand side of the equation because the total volume is the volume of ultrafiltrate and the ultrafiltrate concentration of inulin equals its plasma concentration. The fraction of water reabsorbed is V_R/V_T. This is obtained by dividing both sides of Eqn (7.4.11) by V_T and rearranging:

$$\frac{V_R}{V_T} = 1 - \frac{V_{TF}}{V_T} \quad [7.4.13]$$

From Eqn (7.4.12), we see that

$$\frac{V_{TF}}{V_T} = \frac{1}{(TF_{inulin}/P_{inulin})} \quad [7.4.14]$$

Substituting this result into Eqn (7.4.13), we find

$$\frac{V_R}{V_T} = 1 - \frac{1}{(TF/P)_{inulin}} \quad [7.4.15]$$

Thus the fraction of water reabsorbed can be estimated by measuring plasma [inulin] and tubular fluid [inulin] alone. At the end of the proximal tubule $(TF/P)_{inulin} = 3$, so that the fraction of water reabsorbed in the proximal tubule is about $1 - 1/3 = 67\%$.

THE DOUBLE RATIO $(TF/P)_X/(TF/P)_{INULIN}$ IS THE FRACTION OF THE FILTERED LOAD OF *X* REMAINING

The TF/*P* ratio of a substance *x* is ambiguous. Samples of tubular fluid at the end of the proximal tubule show that $(TF/P)_{Na^+} = 1.0$. What does this mean? It could mean that neither Na^+ nor water was reabsorbed in this section of the nephron, or it could mean that they were reabsorbed in the same ratio so that the concentration of Na^+ did not change. The relative movement of substance *x* and water can be determined by taking the double ratio, $(TF/P)_x/(TF/P)_{inulin}$. The conservation equation is as follows:

$$V_{TF}TF_x + R_x = V_T P_x \quad [7.4.16]$$

where TF_x is the concentration of substance *x* in the tubular fluid of volume V_{TF}, R_x is the amount of substance *x* that is reabsorbed, V_T is the total volume of ultrafiltrate, and P_x is the plasma concentration. The fraction of substance remaining in the tubule is given as

$$\text{Fraction remaining} = \frac{V_{TF}TF_x}{V_T P_x} \quad [7.4.17]$$

We can insert the result for V_{TF}/V_T from Eqn (7.4.14) to obtain

$$\text{Fraction remaining} = \frac{(TF/P)_x}{(TF/P)_{inulin}} \quad [7.4.18]$$

MICROPUNCTURE STUDIES SHOW THAT THE PROXIMAL TUBULE REABSORBS TWO-THIRDS OF THE ULTRAFILTRATE

Sampling of the tubular fluid at the end of the proximal tubule shows that **all of the glucose and amino acids are reabsorbed in the proximal tubule and that the remaining fluid is isosmotic with plasma**. This means that the fluid that is reabsorbed in the proximal tubule is also isosmotic. The $(TF/P)_{inulin}$ is about 3 at the end of the proximal tubule. By Eqn (7.4.13), this means that two-thirds of the ultrafiltrate volume has been reabsorbed. The ratio $(TF/P)_{Na^+}$, however, remains about 1.0 at the end of the proximal tubule, so that the double ratio is about 1/3. This is the fraction of filtered Na^+ that remains in the tubule. Thus two-thirds of the filtered Na^+ is also reabsorbed in the proximal tubule. The proximal tubule also absorbs about two-thirds of the filtered load for a variety of other materials including Cl^-, HCO_3^-, and K^+.

THE *PROXIMAL CONVOLUTED TUBULE* CONTAINS MANY TRANSPORT MECHANISMS

GLUCOSE ENTERS BY Na^+-LINKED COTRANSPORT AND LEAVES BY FACILITATED DIFFUSION

Nearly all filtered glucose is reabsorbed in the early proximal tubule. A **s**odium–**g**lucose **l**inked co**t**ransporter **(SGLT)** resides on the microvillus membrane facing the tubular fluid. There are five isoforms of these Na^+–glucose cotransporters. SGLT1 and SGLT2 reside in the proximal tubule, SGLT2 in the proximal convoluted tubule and SGLT1 in the proximal straight tubule. The SGLT1 couples two Na^+ atoms per glucose imported into the cell; with SGLT2 the stoichiometry is 1 Na^+:1 glucose. The energy cost for glucose entry is paid by Na^+ running from a high electrochemical potential to a lower one. The low electrochemical potential of Na^+ inside the cell is maintained by the **Na,K-ATPase** located on the basolateral membrane of the proximal tubule cells. Glucose that enters the cell leaves it by facilitated diffusion on carriers called GLUT. The early proximal tubule has GLUT2 carriers; the proximal straight tubule has GLUT1 (see Figure 7.4.7).

AMINO ACIDS TRANSPORTERS ARE ON BOTH APICAL AND BASOLATERAL MEMBRANES

Amino acid transport across the renal tubule epithelium reprises that found in the intestines, with some differences. A set of at least five distinct amino acid transporters on the apical membrane bring filtered amino acids into the proximal tubule cell. This set of amino acid transporters are necessary because of the heterogeneity of the amino acids that must be reabsorbed. One of these carries neutral amino acids; a second carries the anionic amino acids; another transports cationic amino acids and cystine; another carries proline and hydroxyproline; another carries proline, glycine, and alanine. Some of these carriers require Na^+ as a cotransported ion (**secondary active transport**), and some do not (**facilitated diffusion**). Another set of carriers carry the amino acids across the basolateral membrane. Some of these carriers are identical to those on the apical membrane, and some are distinct (see Figure 7.4.8).

Na^+ COTRANSPORTERS CARRY ORGANIC ANIONS

The apical membrane contains a number of other transporters that bring organic anions into the proximal tubule cell by secondary active transport with Na^+ energetics driving the inward movement of the anion. Examples include phosphate, lactate, citrate, succinate, and acetate. As shown by the renal titration curve, the urine generally contains some phosphate, so not all of the phosphate is reabsorbed. Lactate, on the other hand, is completely reabsorbed from the urine. Specific carrier proteins carry lactate and phosphate out of the cell and into the blood flowing

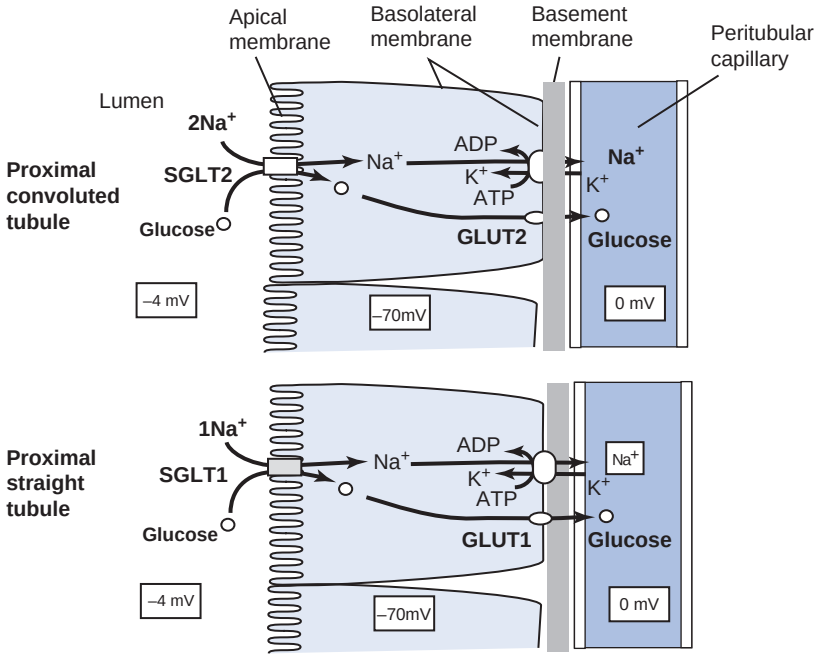

FIGURE 7.4.7 Mechanism of glucose absorption in the proximal convoluted tubule and proximal straight tubule. See text for details.

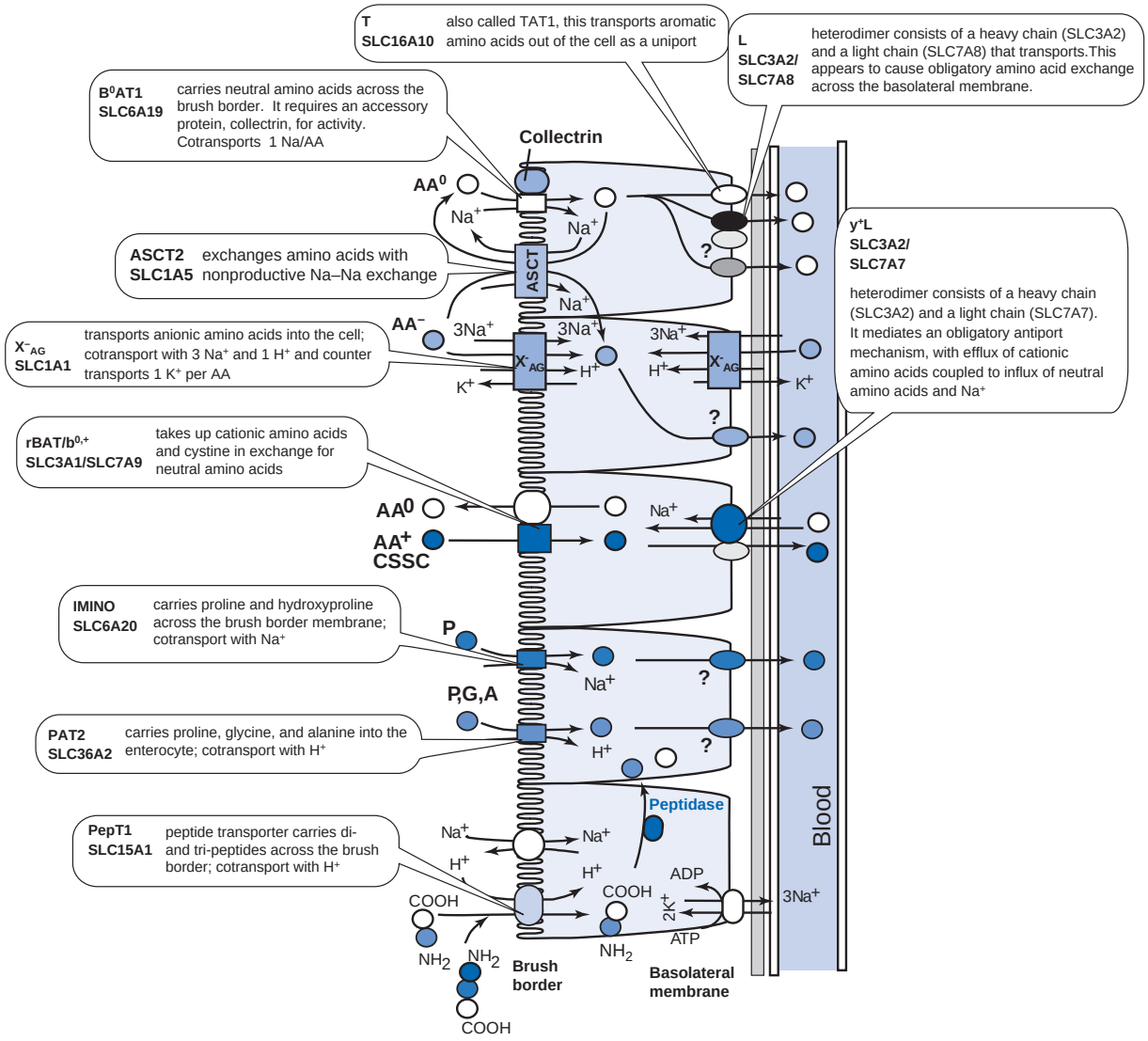

FIGURE 7.4.8 Mechanism of amino acid transport in the proximal tubule. See text for details.

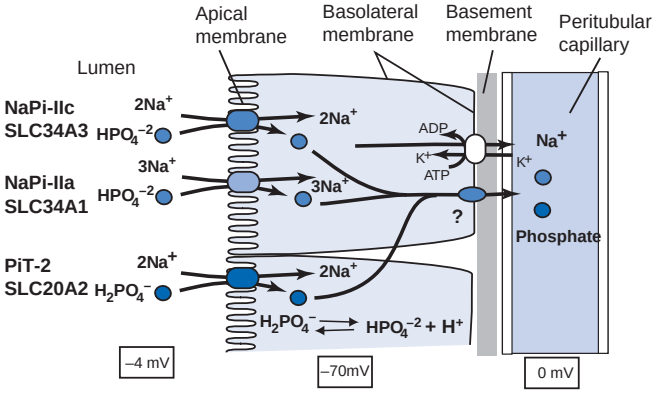

FIGURE 7.4.9 Mechanism of phosphate absorption in proximal tubule cells. Three distinct phosphate carriers on the brush border membrane have been identified. All use Na^+ co-transport and thus are powered by the Na^+ gradient established by the basolateral Na-K-ATPase. The NaPi-IIc and PiT-2 transporters both use a stoichiometry of 2 Na^+ per phosphate. The Napi-IIa uses3 Na^+ per phosphate. All three are located in the proximal convoluted tubule.

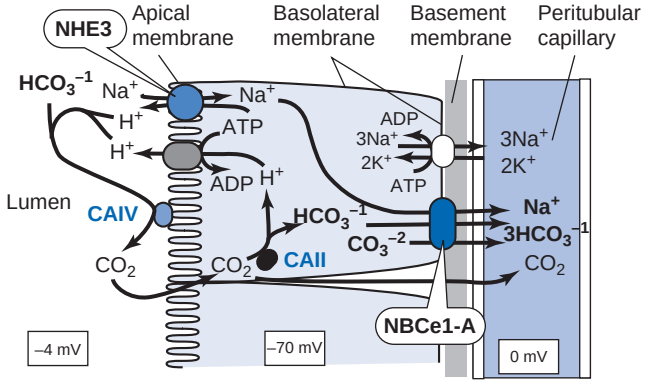

FIGURE 7.4.10 Mechanism of HCO_3^- absorption in the proximal tubule. Filtered HCO_3^- combines with H^+ secreted into the tubule by Na–H exchange over **NHE3**, or, less importantly, by an H^+ pump. This is converted to CO_2 by an apical membrane-bound carbonic anhydrase (CAIV). CO_2 from the luminal fluid or peritubular blood is converted to HCO_3^- and H^+ in the cytosol by the aid of a soluble carbonic anhydrase (CAII). A Na–HCO_3^- cotransporter, **NBCe1-A**, transports one Na^+ along with the equivalent of three HCO_3^- (one HCO_3^{-1} and one CO_3^{-2}). In this way, Na^+ and HCO_3^- are removed from the filtrate and Na^+ and HCO_3^{-1} appear in the blood.

through the peritubular capillaries. These carriers employ facilitated diffusion (see Figure 7.4.9).

BICARBONATE ABSORPTION IS LINKED TO ACID SECRETION

The absorption of HCO_3^- begins with H^+ secretion into the lumen through two mechanisms: **Na^+–H^+ exchange** and a **H^+-ATPase pump**. The secreted H^+ along with filtered HCO_3^- is converted to H_2O and CO_2 by the action of **carbonic anhydrase** on the apical membrane (**CAIV**). The CO_2 is transported into the cell and equilibrates with peritubular capillary CO_2. In the cell, CO_2 is converted to H^+ and HCO_3^- by a cytosolic carbonic anhydrase (**CAII**). HCO_3^- can dissociate to form H^+ and CO_3^{-2}. The sodium–bicarbonate exchanger (NBCe1-B) transports HCO_3^- and CO_3^{-2} out of the cell with a cotransport of Na^+. The H^+ formed from the hydration reaction of CAII feeds into the $Na^+ - H^+$ exchanger and the H^+-ATPase pump. The result of the entire operation is that Na^+ and HCO_3^- disappear from the lumen and Na^+ and HCO_3^- appear in the peritubular blood. The HCO_3^- that appears in the blood is not the same as that which disappeared from the lumen, but the net effect is the same as if it were (see Figure 7.4.10).

THE PROXIMAL CONVOLUTED TUBULE PASSIVELY REABSORBS UREA

Urea is a small nitrogenous compound (molecular weight is 60) that is the main end product of protein catabolism in mammals (see Chapter 2.11). It is made predominantly in the liver from ammonia and bicarbonate and is one of the main components of urine. The rate of synthesis varies from 300 to 600 mmol/day depending on the protein intake. All of this urea eventually finds its way into the urine. Because urea makes up a large part of the obligatory solute excretion, its osmotic pressure requires significant volumes of water to carry the urea. Urea passively crosses biological membranes, but its permeability is low because of its low solubility in the lipid bilayer. Some cells speed up this process through **urea transporters**, which move urea by facilitated diffusion. Urea is passively reabsorbed in the proximal tubule, but its route of transport is not clear. Urea transporters have not yet been identified for the proximal tubule. SGLT1 can transport urea via Na–urea transport.

WATER FOLLOWS THE OSMOTIC PRESSURE GRADIENT THROUGH WATER CHANNELS

Water reabsorption is by osmosis through water channels in the membrane. These water channels consist of a family of proteins called **aquaporin**. At least seven different aquaporin isoforms are expressed in the kidney. The proximal tubule has abundant **AQP1** on the apical and basolateral membranes and **AQP7** on the apical membrane of the late proximal tubule.

The blood that flows through the peritubular capillaries that surround the proximal tubules originates from the efferent arterioles of cortical nephrons. This blood has passed through the glomerulus and has had a protein-free filtrate abstracted from it. Thus the protein concentration in the efferent arterioles is increased by removal of 20% of the plasma volume while leaving the proteins behind. Therefore, the peritubular capillaries contain plasma with a higher oncotic pressure. As Na^+ is reabsorbed with other solutes, the concentration of osmolytes in the spaces between the cells increases, causing a local increase in the osmotic pressure in this space. Water moves in response to the high oncotic pressure of the peritubular capillaries and the slight hyperosmolarity of the lateral intracellular space, so that water flows across the basolateral membrane into the lateral

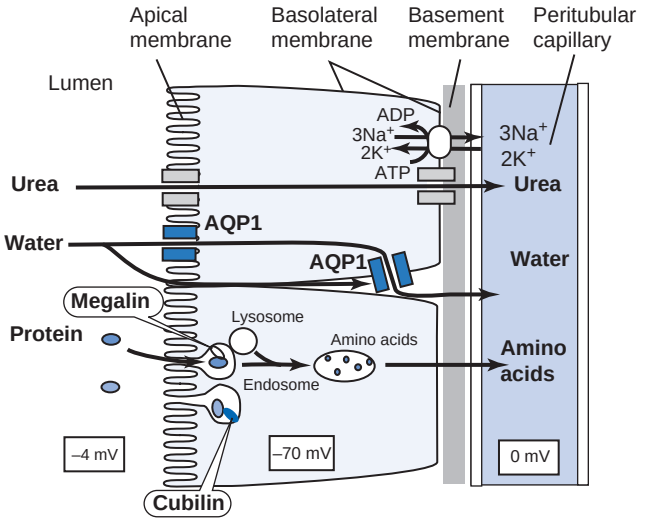

FIGURE 7.4.11 Mechanism of urea, water, and protein reabsorption in the proximal tubule. See text for details.

intracellular space and into the interstitial space surrounding the capillaries, and from there into the peritubular capillaries. As water moves from the cell, it concentrates the cell contents so that the osmotic gradient is transferred to the apical membrane. Water moves from the tubular fluid into the cell in response to this gradient. The net effect is water reabsorption from the tubular fluid into the peritubular capillaries, caused by the increased oncotic pressure of the capillary blood and the active reabsorption of Na^+ and other solutes (see Figure 7.4.11).

THE PROXIMAL TUBULE REABSORBS FILTERED PROTEINS BY ENDOCYTOSIS

The concentration of proteins in the ultrafiltrate is small but not zero because the sieving coefficient is not exactly zero but is some small value that depends on the size of the protein. Normal plasma albumin concentrations are 3.5–5.0 g dL^{-1}, whereas the concentration in the ultrafiltrate is about 1–3 mg dL^{-1}, giving a sieving coefficient of about 0.0003–0.0008. Thus about 1.8 g of albumin are filtered per day. In addition, a variety of low molecular weight proteins and polypeptide hormones are filtered by the kidney and subsequently destroyed by it. These filtered proteins and polypeptides are taken up by an extensive network of coated pits in the proximal tubule. Two broad-spectrum binding proteins, megalin and cubilin, recognize and bind filtered proteins and direct them into the coated pits. Kidney epithelial cells take up the proteins by endocytosis, and the resulting endosome fuses with a lysosome, with its content of hydrolytic enzymes, and the proteins are hydrolyzed to their constituent amino acids. The amino acids are then reabsorbed into the peritubular capillary blood (see Figure 7.4.11).

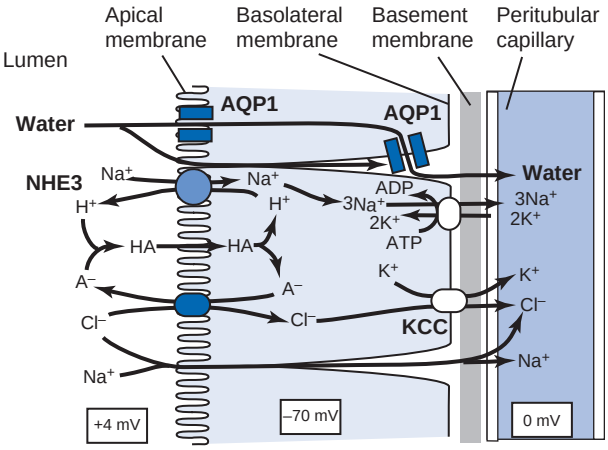

FIGURE 7.4.12 Mechanism of water and NaCl reabsorption in the late proximal tubule. By the time the tubular fluid gets to the late proximal tubule, a large fraction of the filtered water and Na^+ has already been reabsorbed. In the early proximal tubule, Na^+ reabsorption is preferentially accompanied by HCO_3^- reabsorption. This concentrates the Cl^- that is left behind so that its concentration exceeds that of the peritubular blood. Transport of Cl^- occurs through a cellular route and a paracellular route. In the cellular route, Cl^- enters the cell in exchange for an anion, which could be OH^-, formate (HCO_2^-), oxalate, HCO_3^-, or sulfate. This process occurs in parallel with a Na^+–H^+ exchanger so that the net effect is entry of NaCl into the cell. Chloride exits the cell passively because the interior of the cell is sufficiently negative to overcome the unfavorable difference in Cl^- concentration between the cell and the peritubular fluid. Some of the chloride is believed to be carried by K–Cl cotransport channels, KCC, KCC1, KCC3 and KCC4 are expressed in the kidney. The Na^+,K^+-ATPase pumps the Na^+ out of the cell at the basolateral membrane. NaCl also travels through the junctions between the cells in the paracellular route. This draws water from the tubular fluid by osmosis, and some of this water goes through the paracellular path. The osmotic pressure difference between the peritubular fluid and the tubular fluid is small, on the order of 2–6 mOsM. This is sufficient to drive water reabsorption.

ABSORPTION OF WATER AND SALT ACROSS THE LATE PROXIMAL TUBULE

The transport of Na^+, Cl^-, and water is different in the late proximal tubule than it is in the early proximal tubule. In the early proximal tubule, Na^+ is reabsorbed preferentially with HCO_3^-. In the late proximal tubule, Na^+ is reabsorbed with Cl^-. As Na^+ gets absorbed along with the amino acids and glucose and HCO_3^- in the early proximal tubule, the [Cl^-] in the tubular fluid increases. This higher [Cl^-] drives Cl^- diffusion through the so-called tight junctions between the cells at the apical aspect. This creates a lumen positive potential that favors Na^+ reabsorption. Chloride enters the tubule cells in part in exchange for secreted anions. The mechanism is illustrated in Figure 7.4.12.

SUMMARY

The filtered load of water and many nutrients is enormous. Most of the filtered material does not show up in the final urine because it is reabsorbed back into the peritubular capillaries that perfuse the nephron. The overall handling of materials is made

evident from renal titration curves. Because inulin is freely filtered but not reabsorbed or secreted, its renal excretion rate is linear with its plasma concentration. This means that the clearance of inulin is independent of its plasma concentration. In contrast, no glucose appears in the urine until the plasma concentration exceeds its renal threshold, which is substantially higher than normal plasma glucose concentrations. Thus normally the kidney does not participate in regulating plasma glucose concentration. Normally the glucose clearance is zero because it is avidly reabsorbed in the proximal tubule. The renal titration curve for phosphate is similar to that of glucose except that the renal threshold is near the normal plasma concentration for phosphate. Normally there is some phosphate excretion, and if plasma phosphate levels rise, more phosphate spills over into the urine. The renal titration curve for para-amino hippuric acid (PAH) shows that it is secreted by the kidney. Because it is so avidly secreted, the clearance of PAH approximates the renal plasma flow. Creatinine is a waste product of muscle metabolism whose clearance approximates the GFR.

The ratio of tubular fluid concentration of inulin to plasma concentration of inulin can be used to estimate absorption of water. The double ratio $[TF_x/P_x]/[TF_{inulin}/P_{inulin}]$ can be used to estimate the relative handling of water and material.

About two-thirds of filtered water is absorbed in the proximal tubule, along with all of the filtered glucose and amino acids and about two-thirds of the filtered Na^+, Cl^-, and HCO_3^-. Glucose and amino acids are absorbed by secondary active transport at the apical membrane of proximal tubule cells and by facilitated transport across the basolateral membrane into the peritubular capillary blood. The secondary active transport requires Na^+ cotransport down its electrochemical gradient. The electrochemical gradient for Na^+ is maintained by a basolateral Na,K-ATPase pump. Urea and water are reabsorbed passively by specific carriers. HCO_3^- reabsorption is linked to H^+ secretion powered by Na^+-H^+ exchange and an apical H^+-ATPase pump. The small amount of filtered protein is reabsorbed by endocytosis.

REVIEW QUESTIONS

1. Why is the clearance of inulin independent of its plasma concentration?
2. Under what conditions is the clearance of PAH equal to the effective renal plasma flow, ERPF?
3. The renal threshold is usually less than $T_m/(GFR \times \Theta)$. Why?
4. How do you calculate the rate of reabsorption or rate of secretion?
5. What does the ratio TF_{inulin}/P_{inulin} signify? What is its value at the end of the proximal tubule?
6. What is the significance of $(TF/P)_x/(TF/P)_{inulin}$? What is this double ratio for Na at the end of the proximal tubule? What is it for glucose?
7. How are amino acids reabsorbed from the proximal tubule? Where do they go?
8. How is glucose reabsorbed from the proximal tubule?
9. How is HCO_3^- reabsorbed from the proximal tubule?