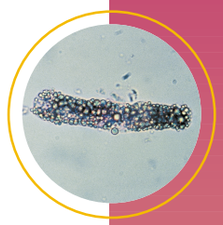
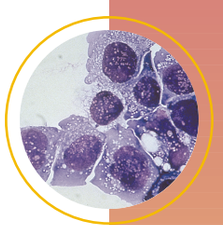
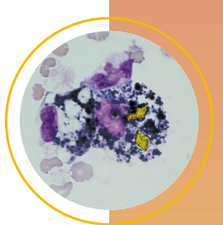
CHAPTER 2







Renal Function

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

1. Identify the components of the nephron, kidney, and excretory system.
2. Trace the flow of blood through the nephron, and state the physiologic functions that occur.
3. Describe the process of glomerular ultrafiltration.
4. Discuss the functions and regulation of the renin-angiotensin-aldosterone system.
5. Differentiate between active and passive transport in relation to renal concentration.
6. Explain the function of antidiuretic hormone in the concentration of urine.
7. Describe the role of tubular secretion in maintaining acid-base balance.
8. Identify the laboratory procedures used to evaluate glomerular filtration, tubular reabsorption and secretion, and renal blood flow.
9. Discuss the advantages and disadvantages in using urea, inulin, creatinine, beta$_2$ microglobulin, and radionucleotides to measure glomerular filtration.
10. Given hypothetic laboratory data, calculate a creatinine clearance and determine whether the result is normal.
11. Discuss the clinical significance of the creatinine clearance test.
12. Define osmolarity and discuss its relationship to urine concentration.
13. Describe the basic principles of clinical osmometers.
14. Given hypothetic laboratory data, calculate a free-water clearance and interpret the result.
15. Given hypothetic laboratory data, calculate a PAH clearance and relate this result to renal blood flow.
16. Describe the relationship of urinary ammonia and titratable acidity to the production of an acidic urine.

KEY TERMS

active transport
aldosterone
maximal reabsorptive capacity
osmolarity
passive transport
podocytes
renal threshold
renal tubular acidosis
renin
renin-angiotension-aldosterone system
titratable acidity
tubular reabsorption
tubular secretion
vasopressin

This chapter reviews nephron anatomy and physiology and discusses its relationship to urinalysis and renal function testing. A section on laboratory assessment of renal function follows.

Renal Physiology

Each kidney contains approximately 1 to 1.5 million functional units called **nephrons**. As shown in Figure 2–1, the human kidney contains two types of nephrons. Cortical nephrons, which make up approximately 85% of nephrons, are situated primarily in the cortex of the kidney. Juxtamedullary nephrons have longer Henle's loops that extend deep into the medulla of the kidney.

The ability of the kidneys to selectively clear waste products from the blood and simultaneously maintain the body's essential water and electrolyte balances is controlled in the nephron by the following renal functions: renal blood flow, glomerular filtration, ***tubular reabsorption***, and ***tubular secretion***. The physiology, laboratory testing, and associated pathology of these four functions are discussed in this chapter.

RENAL BLOOD FLOW

The renal artery supplies blood to the kidney. Blood enters the capillaries of the nephron through the **afferent arteriole**. It then flows through the glomerulus and into the **efferent arteriole**. The varying sizes of these arterioles help to create the hydrostatic pressure differential important for glomerular filtration and also to maintain consistency of glomerular capillary pressure and renal blood flow within the glomerulus. Notice the smaller size of the efferent arteriole in Figure 2–2. This increases the glomerular capillary pressure.

Before returning to the renal vein, blood from the efferent arteriole enters the **peritubular capillaries** and the **vasa recta** and flows slowly through the cortex and medulla of the kidney close to the tubules. The peritubular capillaries surround the proximal and distal convoluted tubules, providing for the immediate reabsorption of essential substances from the fluid in the **proximal convoluted tubule** and final adjustment of the urinary composition in the **distal convoluted tubule**. The vasa recta are located adjacent to the ascending and descending **loop of Henle** in juxtamedullary nephrons. In this area, the major exchanges of water and salts take place between the blood and the **medullary interstitium**. The exchange of water and salts between the blood in the vasa recta and the medullary interstitium maintains the **osmotic gradient** (salt concentration) in the medulla that is necessary for renal concentration.

Based on an average body size of 1.73 m^2 of surface, the total renal blood flow is approximately 1200 mL/min and the total **renal plasma flow** ranges from 600 to 700 mL/min. Normal values for renal blood flow and renal function tests depend on body size. When dealing with sizes that vary greatly from the average 1.73 m^2 of body surface, a correction must be calculated to determine whether

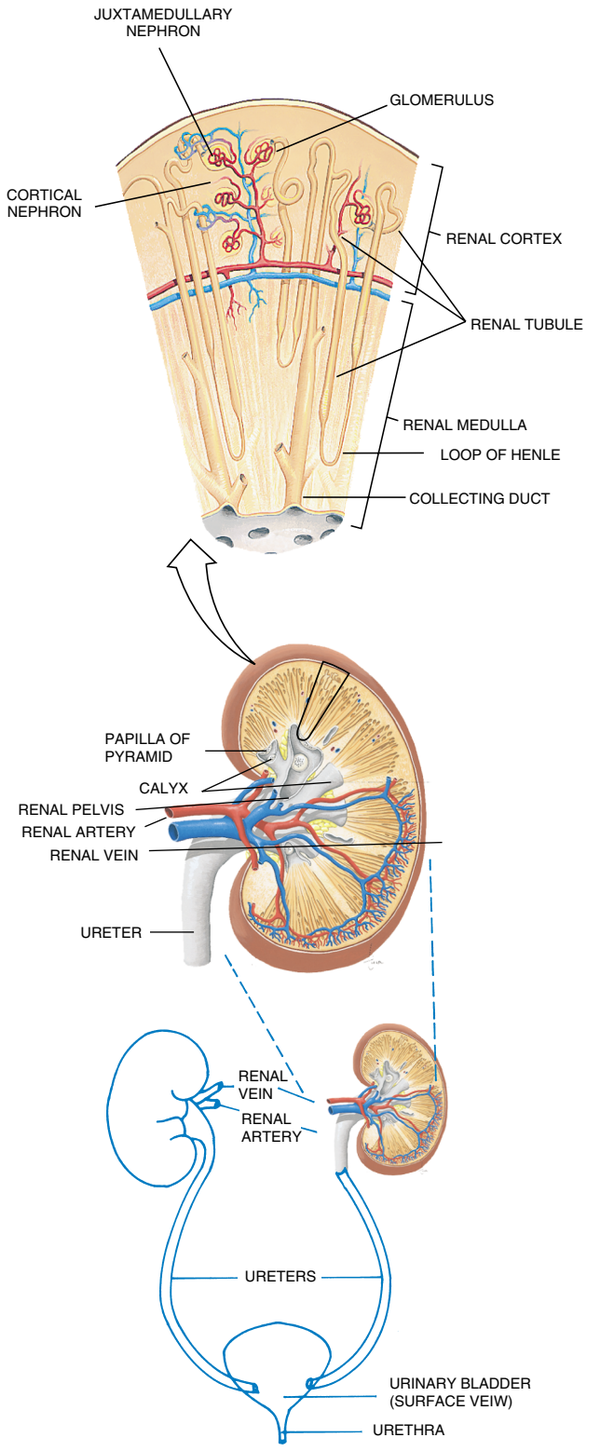


FIGURE 2–1 The relationship of the nephron to the kidney and excretory system. (From Scanlon, VC, and Sanders, T: Essentials of Anatomy and Physiology, ed 3. FA Davis, Philadelphia, 1999, p 405, with permission.)

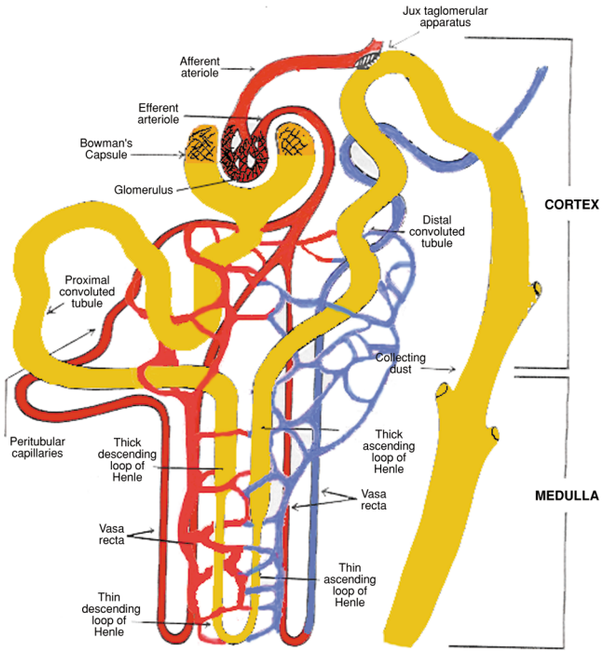


FIGURE 2–2 The nephron and its component parts.

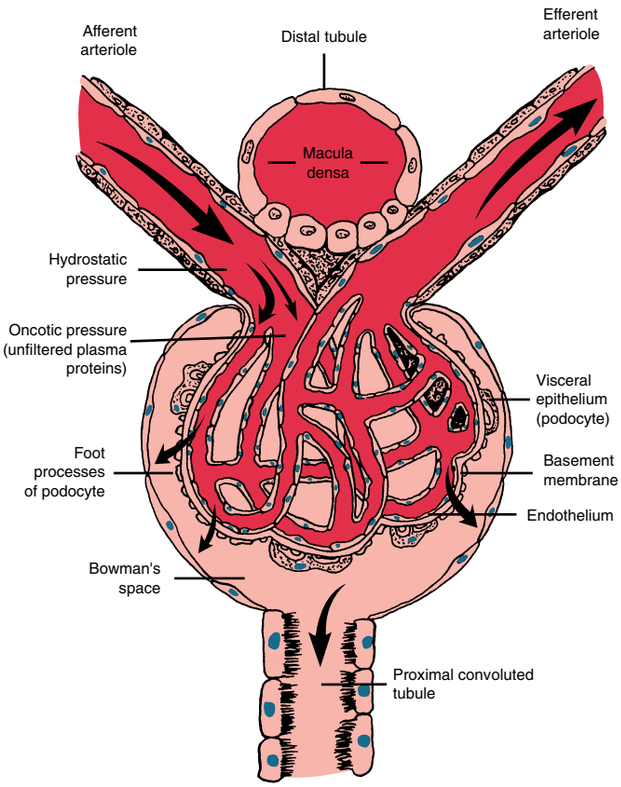


FIGURE 2–3 Factors affecting glomerular filtration in the renal corpuscle.

the observed measurements represent normal function. This calculation is covered in the discussion on tests for **glomerular filtration rate** later in this chapter. Variations in normal values also have been published for different age groups and should be considered when evaluating renal function studies.

GLOMERULAR FILTRATION

The **glomerulus** consists of a coil of approximately eight capillary lobes referred to collectively as the capillary tuft. It is located within **Bowman's capsule** and forms the beginning of the renal tubule. Although the glomerulus serves as a nonselective filter of plasma substances with molecular weights of less than 70,000, several factors influence the actual filtration process. These include the cellular structure of the capillary walls and Bowman's capsule, **hydrostatic** and **oncotic pressures**, and the feedback mechanisms of the ***renin-angiotensin-aldosterone system***. Figure 2–3 provides a diagrammatic view of the glomerular areas influenced by these factors.

Plasma filtrate must pass through three cellular layers: the capillary wall membrane, the basement membrane (basal lamina), and the visceral epithelium of Bowman's capsule. The endothelial cells of the capillary wall differ from those in other capillaries by containing pores and are referred to as fenestrated. The pores increase capillary permeability but do not allow the passage of large molecules and blood cells. Further restriction of large molecules occurs as the filtrate passes through the basement membrane and the thin membranes covering the filtration slits formed by the intertwining foot processes of the ***podocytes*** of the inner layer of Bowman's capsule (see Fig. 2–3).

As mentioned previously, the presence of hydrostatic pressure resulting from the smaller size of the efferent arteriole and the glomerular capillaries enhances filtration. This pressure is necessary to overcome the opposition of pressures from the fluid within Bowman's capsule and the oncotic pressure of unfiltered plasma proteins in the glomerular capillaries. By increasing or decreasing the size of the afferent arteriole, an autoregulatory mechanism within the kidney maintains the glomerular blood pressure at a relatively constant rate regardless of fluctuations in systemic blood pressure. Dilation of the afferent arterioles when blood pressure drops prevents a marked decrease in blood flowing through the kidney, thus preventing an increase in the blood level of toxic waste products.

The renin-angiotensin-aldosterone system controls the regulation of the flow of blood to and within the kidney. The system responds to changes in blood pressure and plasma sodium content that are monitored by the **juxtaglomerular apparatus**, which consists of the juxtaglomerular cells in the afferent arteriole and the **macula densa** of the distal convoluted tubule (see Fig. 2–2). Low plasma sodium content decreases water retention within the circulatory system, resulting in a decreased overall blood volume

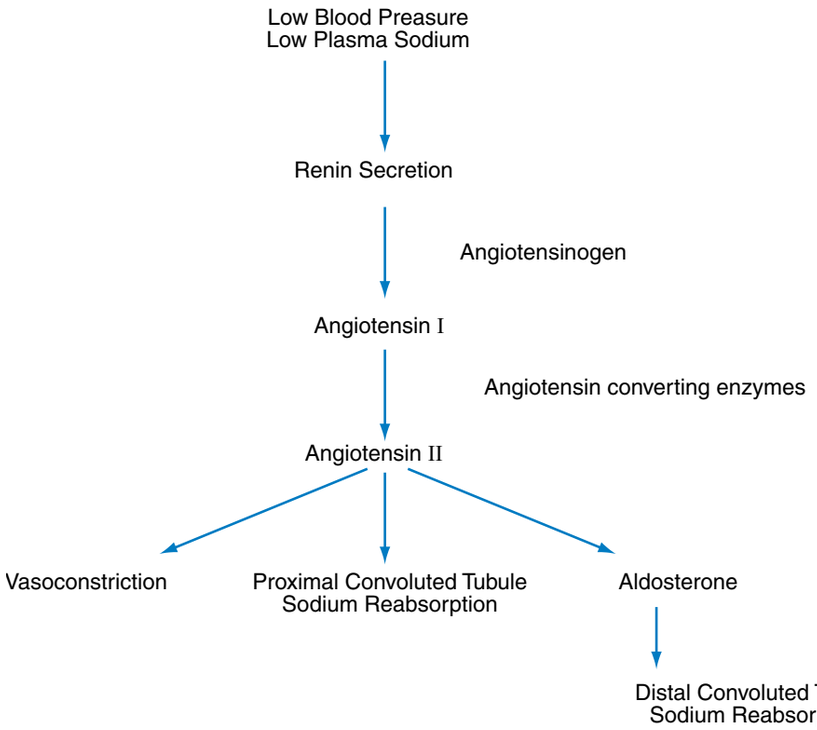


FIGURE 2–4 Actions of the renin-angiotensin-aldosterone system.

and subsequent decrease in blood pressure. When the juxtaglomerular apparatus senses such changes, a cascade of reactions within the renin-angiotensin-aldosterone system occurs (Figure 2–4). ***Renin***, an enzyme produced by the juxtaglomerular apparatus, is secreted and reacts with the bloodborne substrate angiotensinogen to produce the inert hormone angiotensin I. As angiotensin I passes through the lungs, converting enzymes change it to the active form angiotensin II. Angiotensin II corrects renal blood flow in the following three ways: causing vasoconstriction of the renal arterioles, stimulating reabsorption of sodium in the proximal convoluted tubule, and triggering the release of the sodium-retaining hormone ***aldosterone*** from the adrenal cortex. As systemic blood pressure and plasma sodium content increase, the secretion of renin decreases. Therefore, the actions of angiotensin II produce a constant pressure within the nephron.

As a result of the above glomerular mechanisms, every minute approximately 2 to 3 million glomeruli filter approximately 120 mL of water-containing low-molecular-weight substances. Because this filtration is nonselective, the only difference between the compositions of the filtrate and the plasma is the absence of plasma protein, any protein-bound substances, and cells. Analysis of the fluid as it leaves the glomerulus shows the filtrate to have a specific gravity of 1.010 and confirms that it is chemically an ultrafiltrate of plasma. This information provides a useful baseline for evaluating the renal mechanisms involved in converting the plasma ultrafiltrate into the final urinary product.

TUBULAR REABSORPTION

The body cannot lose 120 mL of water-containing essential substances every minute. Therefore, when the plasma ultrafiltrate enters the proximal convoluted tubule, the kidney, through cellular transport mechanisms, begins reabsorbing these essential substances and water (Table 2–1).

The cellular mechanisms involved in tubular reabsorption are termed ***active*** and ***passive transport***. For active transport to occur, the substance to be reabsorbed must combine with a carrier protein contained in the membranes of the renal tubular cells. The electrochemical energy created by this interaction transfers the substance across the cell membranes and back into the bloodstream. Active transport is responsible for the reabsorption of glucose, amino acids, and salts in the proximal convoluted tubule and the reabsorption of chloride in the ascending loop of Henle and sodium in the distal convoluted tubule.

Passive transport is the movement of molecules across a membrane as a result of differences in their concentration or electrical potential on opposite sides of the membrane. These physical differences are called gradients. Passive reabsorption of water takes place in all parts of the nephron except the ascending loop of Henle, the walls of which are impermeable to water. Urea is passively reabsorbed in the proximal convoluted tubule and the ascending loop of Henle, and passive reabsorption of sodium accompanies the active transport of chloride in the ascending loop of Henle.

TABLE 2–1 **Tubular Reabsorption**

	Substance	Location
Active transport	Glucose, amino acids, and salts	Proximal convoluted tubule
	Chloride	Ascending loop of Henle
	Sodium	Proximal and distal convoluted tubule
Passive transport	Water	Proximal convoluted tubule, descending loop of Henle, and collecting tubules
	Urea	Proximal convoluted tubule and ascending loop of Henle
	Sodium	Ascending loop of Henle

Active transport, like passive transport, can be influenced by the concentration of the substance being transported. When the plasma concentration of a substance that is normally completely reabsorbed reaches an abnormally high level, the filtrate concentration exceeds the ***maximal reabsorptive capacity*** **(Tm)** of the tubules, and the substance begins appearing in the urine. The plasma concentration at which active transport stops is termed the ***renal threshold***. For glucose, the renal threshold is 160 to 180 mg/dL, and glucose appears in the urine when the plasma concentration reaches this level. Knowledge of the renal threshold and the plasma concentration can be used to distinguish between excess solute filtration and renal tubular damage. For example, glucose appearing in the urine of a person with a normal blood glucose level is the result of tubular damage and not diabetes mellitus.

Active transport of more than two-thirds of the filtered sodium out of the proximal convoluted tubule is accompanied by the passive reabsorption of an equal amount of water. Therefore, as can be seen in Figure 2–5, the fluid leaving the proximal convoluted tubule still maintains the same concentration as the ultrafiltrate.

Renal concentration begins in the descending and ascending loop of Henle, where the filtrate is exposed to the high osmotic gradient of the renal medulla. Water is removed by osmosis in the descending loop of Henle, and sodium and chloride are reabsorbed in the ascending loop of Henle. Excessive reabsorption of water as the filtrate passes through the highly concentrated medulla is prevented by the water-impermeable walls of the ascending loop. This selective reabsorption process is called the **countercurrent mechanism** and serves to maintain the osmotic gradient of the medulla. The sodium and chloride leaving the filtrate in the ascending loop prevent dilution of the medullary interstitium by the water reabsorbed from the descending loop. Maintenance of this osmotic gradient is essential for the final concentration of the filtrate when it reaches the **collecting duct**.

In Figure 2–5, the actual concentration of the filtrate leaving the ascending loop of Henle is quite low owing to the reabsorption of salt and not water in that part of the tubule. Reabsorption of sodium continues in the distal convoluted tubule, but it is now under the control of the hormone aldosterone, which regulates reabsorption in response to the body's need for sodium.

The final concentration of the filtrate through the reabsorption of water begins in the late distal convoluted tubule and continues in the collecting duct. Reabsorption depends on the osmotic gradient in the medulla and the hormone ***vasopressin*** (antidiuretic hormone **[ADH]**). One would expect that as the dilute filtrate in the collecting duct comes in contact with the higher osmotic concentration of the medullary interstitium, passive reabsorption of water would occur. However, the process is controlled by the presence or absence of ADH, which renders the walls of the distal convoluted tubule and collecting duct permeable or impermeable to water. A high level of ADH increases permeability, resulting in increased reabsorption of water and a low-volume, concentrated urine. Likewise, absence of ADH renders the walls impermeable to water, resulting in a large volume of dilute urine. Just as the production of aldosterone is controlled by the body's sodium concentration, production of ADH is determined by the state of body hydration. Therefore, the chemical balance in the body is actually the final determinant of urine volume and concentration. The concept of ADH control can be summarized in the following manner:

↑Body Hydration = ↓ADH = ↑Urine Volume
↓Body Hydration = ↑ADH = ↓Urine Volume

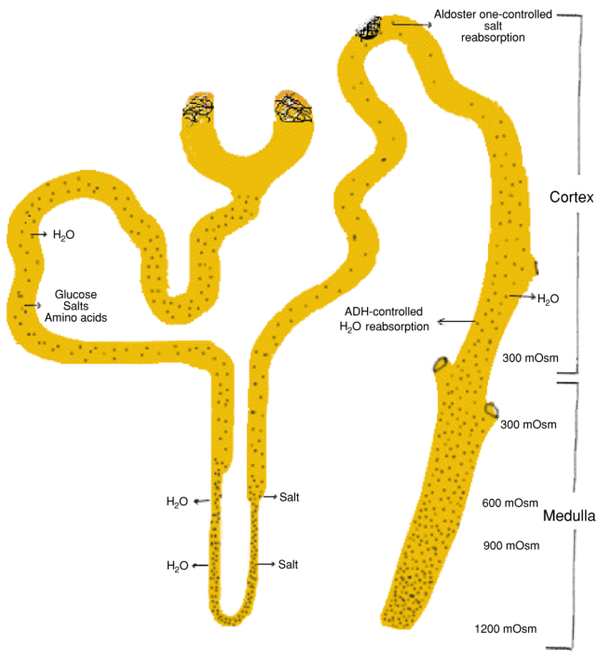


FIGURE 2–5 Renal concentration.

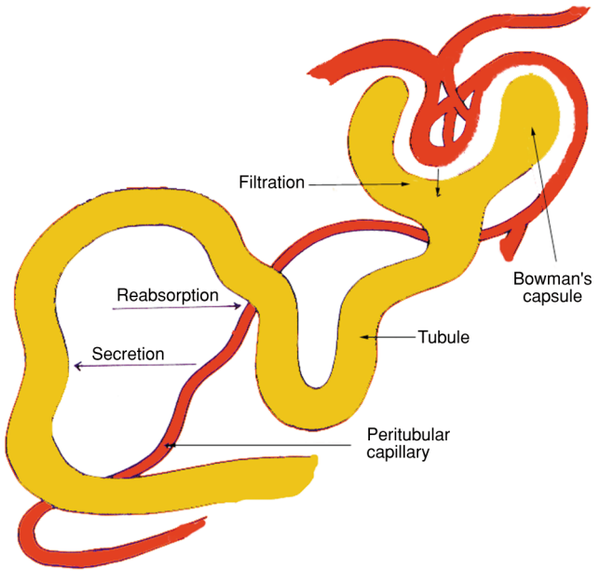


FIGURE 2–6 The movement of substances in the nephron.

TUBULAR SECRETION

In contrast to tubular reabsorption, in which substances are removed from the glomerular filtrate and returned to the blood, tubular secretion involves the passage of substances from the blood in the peritubular capillaries to the tubular filtrate (Figure 2–6). Tubular secretion serves two major functions: elimination of waste products not filtered by the glomerulus and regulation of the acid-base balance in the body through the secretion of hydrogen ions.

Many foreign substances, such as medications, cannot be filtered by the glomerulus because they are bound to plasma proteins. However, when these protein-bound substances enter the peritubular capillaries, they develop a strong affinity for the tubular cells and dissociate from their carrier proteins, which results in their transport into the filtrate by the tubular cells. The major site for removal of these nonfiltered substances is the proximal convoluted tubule.

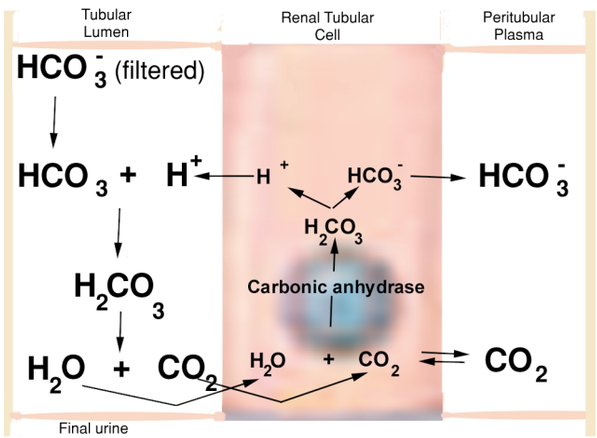


FIGURE 2–7 Reabsorption of filtered bicarbonate.

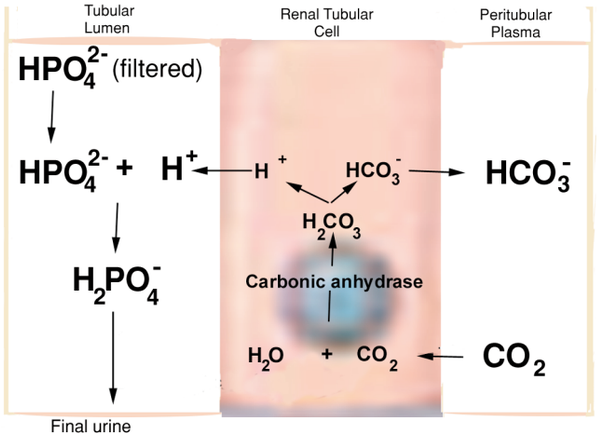


FIGURE 2–8 Excretion of secreted hydrogen ions combined with phosphate.

To maintain the normal blood pH of 7.4, the blood must buffer and eliminate the excess acid formed by dietary intake and body metabolism. The buffering capacity of the blood depends on bicarbonate (HCO_3^-) ions, which are readily filtered by the glomerulus and must be expediently returned to the blood to maintain the proper pH. As shown in Figure 2–7, the secretion of hydrogen ions by the renal tubular cells into the filtrate prevents the filtered bicarbonate from being excreted in the urine and causes the return of a bicarbonate ion to the plasma. This process provides for almost 100 percent reabsorption of filtered bicarbonate and occurs primarily in the proximal convoluted tubule.

The actual excretion of excess hydrogen ions also depends on tubular secretion. Figures 2–8 and 2–9 are diagrams of the two primary methods for hydrogen ion excretion in the urine. In Figure 2–8, the secreted hydrogen ion combines with a filtered phosphate ion instead of a bicarbonate ion and is excreted rather than reabsorbed. Additional excretion of hydrogen ions is accomplished through their reaction with ammonia produced and secreted by the cells of the distal convoluted tubule (see Fig. 2–9). The resulting ammonium ion is excreted in the urine.

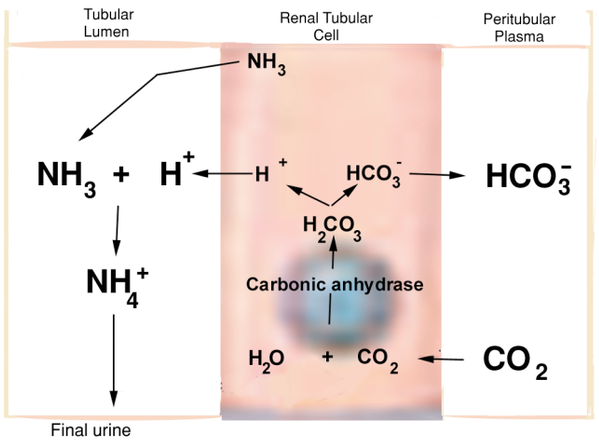


FIGURE 2–9 Excretion of secreted hydrogen ions combined with ammonia produced by the tubules.

All three of these processes are occurring simultaneously at rates determined by the acid-base balance in the body. A disruption in these secretory functions can result in **metabolic acidosis** or ***renal tubular acidosis***, the inability to produce an acid urine.

Renal Function Tests

This brief review of renal physiology shows that there are many metabolic functions and chemical interactions to be evaluated through laboratory tests of renal function. In Figure 2–10, the parts of the nephron are related to the laboratory tests used to assess their function.

GLOMERULAR FILTRATION TESTS

The standard test used to measure the filtering capacity of the glomeruli is the clearance test. As its name implies, a clearance test measures the rate at which the kidneys are able to remove (to clear) a filterable substance from the blood. To ensure that glomerular filtration is being accurately measured, the substance analyzed must be one that is neither reabsorbed nor secreted by the tubules. Other factors to consider in the selection of a clearance test substance include the stability of the substance in urine during a possible 24-hour collection period, the consistency of the plasma level, the substance's availability to the body, and the availability of tests for chemical analysis of the substance.

Clearance Tests

The earliest glomerular filtration tests measured urea because of its presence in all urine specimens and the existence of routinely used methods of chemical analysis. Because approximately 40 percent of the filtered urea is reabsorbed, normal values were adjusted to reflect the reabsorption, and patients were hydrated to produce a urine flow of 2 mL/min to ensure that no more than 40 percent of the urea was reabsorbed. At present, the use of urea as a test substance for glomerular filtration has been replaced by the measurement of either **creatinine**, **inulin**, **beta$_2$ microglobulin**, or radioisotopes.

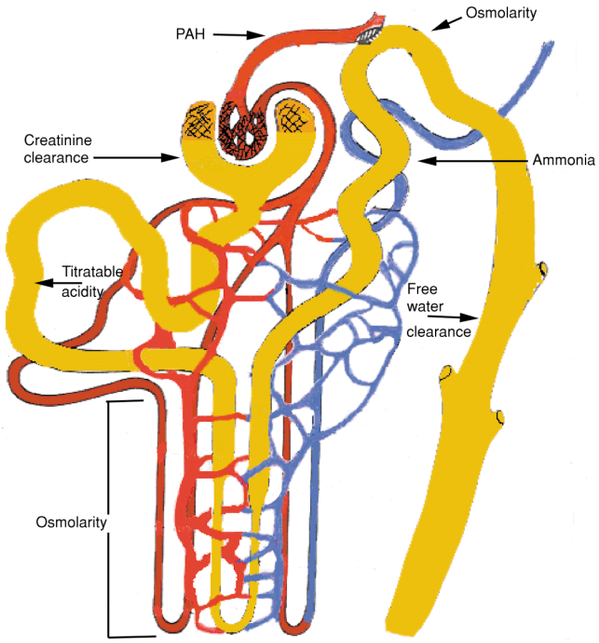


FIGURE 2–10 The relationship of nephron areas to renal function tests.

Inulin, a polymer of fructose, is an extremely stable substance that is not reabsorbed or secreted by the tubules. It is not a normal body constituent, however, and must be infused at a constant rate throughout the testing period. A test that requires an infused substance is termed an **exogenous procedure** and is seldom the method of choice if a suitable test substance is already present in the body (**endogenous procedure**). Therefore, inulin has not been routinely used for glomerular filtration testing.

The development of simplified procedures measuring the plasma disappearance of infused substances, thereby eliminating the need for urine collection, has enhanced interest in exogenous procedures.[3,17] Injection of radionucleotides provides not only a method for determining glomerular filtration through the plasma disappearance of the radioactive material but also enables visualization of the filtration in one or both kidneys.[4]

Good correlation between the glomerular filtration rate and plasma levels of beta$_2$ microglobulin has been demonstrated. Beta$_2$ microglobulin (molecular weight 11,800) dissociates from human leukocyte antigens at a constant rate and is rapidly removed from the plasma by glomerular filtration. Sensitive methods using radioimmunoassay and enzyme immunoassay are available for the measurement of beta$_2$ microglobulin.[14] A rise in the plasma level of beta$_2$ microglobulin has been shown to be a more sensitive indicator of a decrease in glomerular filtration rate than the **creatinine clearance**. However, the test is not reliable in patients who have a history of immunologic disorders or malignancy.[13]

Currently, routine laboratory measurements of glomerular filtration rate employ creatinine as the test substance. Creatinine, a waste product of muscle metabolism that is normally found at a relatively constant level in the blood, provides the laboratory with an endogenous procedure for evaluating glomerular function. The use of creatinine has several disadvantages not found with inulin, and careful consideration should be given to them. Disadvantages are as follows:

1. Some creatinine is secreted by the tubules, and secretion increases as blood levels rise.
2. Chromogens present in human plasma react in the chemical analysis. Their presence, however, may help counteract the falsely elevated rates caused by tubular secretion.
3. Bacteria will break down urinary creatinine if specimens are kept at room temperature for extended periods.[15]
4. A diet heavy in meat consumed during collection of a 24-hour urine specimen will influence the results if the plasma specimen is drawn prior to the collection period.[11]

5. Measurement of creatinine clearance is not a reliable indicator in patients suffering from muscle-wasting diseases.[16]

Because of these drawbacks, abnormal results may be followed up with more sophisticated tests, but the creatinine clearance test can provide the routine clinical laboratory with a method to screen the glomerular filtration rate.

Calculations

By far the greatest source of error in any clearance procedure is the use of improperly timed urine specimens. The importance of using an accurately timed specimen (see Chap. 3) will become evident in the following discussion of the calculations involved in converting isolated laboratory measurements to glomerular filtration rate. The glomerular filtration rate is reported in milliliters per minute; therefore, determining the number of milliliters of plasma from which the clearance substance (creatinine) is completely removed during 1 minute is necessary. To calculate this information, one must know urine volume in milliliters per minute (V), urine creatinine concentration in milligrams per deciliter (U), and plasma creatinine concentration in milligrams per deciliter (P).

The urine volume is calculated by dividing the number of milliliters in the specimen by the number of minutes used to collect the specimen.

EXAMPLE
Calculate the urine volume (V) for a 2-hour specimen measuring 240 mL:

$$2 \text{ hours} \times 60 \text{ minutes} = 120 \text{ minutes}$$

$$\frac{240 \text{ mL}}{120 \text{ minutes}} = 2 \text{ mL/min} \quad V = 2 \text{ mL/min}$$

The plasma and urine concentrations are determined by chemical testing. The standard formula used to calculate the milliliters of plasma cleared per minute (C) is:

$$C = \frac{UV}{P}$$

This formula is derived as follows. The milliliters of plasma cleared per minute (C) times the milligrams per deciliter of plasma creatinine (P) must equal the milligrams per deciliter of urine creatinine (U) times the urine volume in milliliters per minute (V), because all of the filtered creatinine will appear in the urine. Therefore,

$$CP = UV \text{ and } C = \frac{UV}{P}$$

EXAMPLE
Using urine creatinine of 120 mg/dL (U), plasma creatinine of 1.0 mg/dL (P), and urine volume of 1440 mL obtained from a 24-hour specimen (V), calculate the glomerular filtration rate.

$$V = \frac{1440 \text{ mL}}{60 \text{ minutes} \times 24 = 1440 \text{ minutes}} = 1 \text{ mL/min}$$

$$C = \frac{120 \text{ mg/dL (U)} \times 1 \text{ mL/min (V)}}{1.0 \text{ mg/dL (P)}} = 120 \text{ mL/min}$$

By analyzing this calculation and referring to Figure 2–11, one can see that at a 1 mg/dL concentration, each milliliter of plasma contains 0.01 mg creatinine. Therefore, to arrive at a urine concentration of 120 mg/dL (1.2 mg/mL), it would be necessary to clear 120 mL of plasma. Although the filtrate volume is reduced, the amount of creatinine in the filtrate does not change.

Knowing that in the average person (1.73 m^2 body surface) the approximate amount of plasma filtrate produced per minute is 120 mL, it is not surprising that normal creatinine clearance values approach 120 mL/min (men, 107 to 139 mL/min; women, 87 to 107 mL/min). The normal plasma creatinine is 0.5 to 1.5 mg/dL. These normal values take into account variations in size and muscle mass. Values are considerably lower in older people, however, and an adjustment also may have to be made to the calculation when dealing with body sizes that deviate greatly from 1.73 m^2 of surface, such as in children. To adjust a clearance for body size, the formula is:

$$C = \frac{UV}{P} \times \frac{1.73}{A}$$

with A being the actual body size in square meters of surface. The actual body size may be calculated as:

$$\log A = (0.425 \times \log \text{weight}) + (0.725 \times \log \text{height}) - 2.144$$

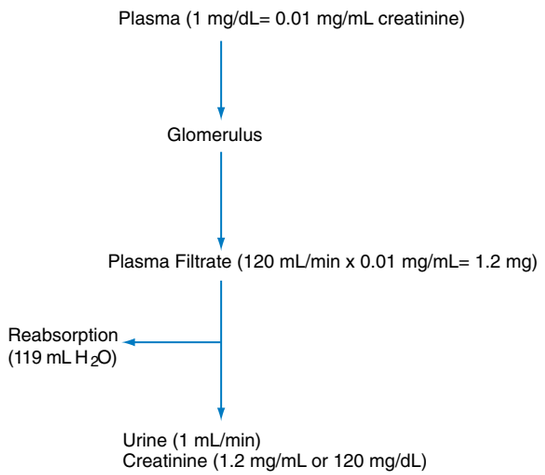


FIGURE 2–11 A diagram representing creatinine filtration and excretion.

or it may be obtained from the nomogram shown in Figure 2–12.

Clinical Significance

When interpreting the results of a creatinine clearance test, one must keep in mind that the glomerular filtration rate is determined not only by the number of functioning nephrons but also by the functional capacity of these nephrons. In other words, even though one-half of the available nephrons may be nonfunctional, a change in the glomerular filtration rate will not occur if the remaining nephrons double their filtering capacity. This is evidenced by those persons who lead normal lives with only one kidney. Therefore, although the creatinine clearance is a frequently requested laboratory procedure, its value does not lie in the detection of early renal disease. Instead, it is used to determine the extent of nephron damage in known cases of renal disease, to monitor the effectiveness of treatment designed to prevent further nephron damage, and to determine the feasibility of administering medications, which can build up to dangerous blood levels if the glomerular filtration rate is markedly reduced.

When medications need to be prescribed prior to waiting for collection of a 24-hour urine specimen, formulas have been developed to predict the creatinine clearance from the serum creatinine. Variables included in the formulas are age, sex, and weight. The most frequently used formula is by Cockcroft and Gault:[6]

$$C_{cr} = \frac{(140 - \text{age})(\text{weight in kilograms})}{72 \times \text{serum creatinine in milligrams per deciliter}}$$

Because the average male weight is approximately 72 kg, the formula can be simplified to:

$$C_{cr} = \frac{140 - \text{age}}{\text{serum creatinine in milligrams per deciliter}}$$

The results are multiplied by 0.85 for female patients. Figures in the formula were obtained by regression analysis of performed and calculated creatinine clearances on 249 male subjects ranging in age from 18 to 92 years.

EXAMPLE

A 50-year-old man has a serum creatinine of 1.1 mg/dL. Calculate his creatinine clearance.

$$\frac{140 - 50}{1.1} = \frac{90}{1.1} = 81.8 \text{ mL/min}$$

This is a reasonable clearance for someone 50 years of age.

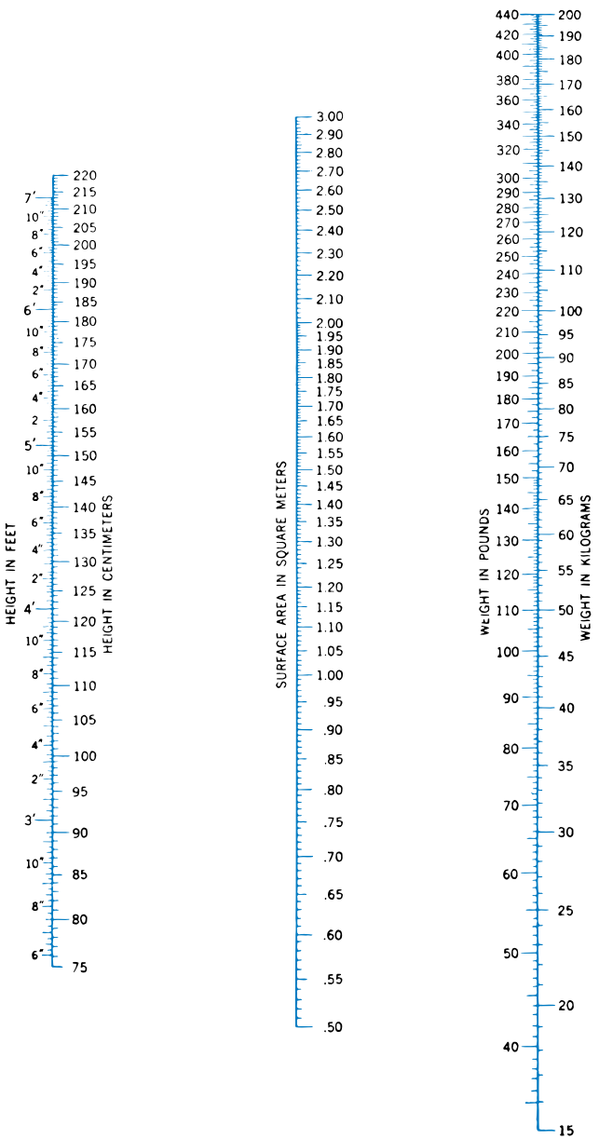


FIGURE 2–12 A nomogram for the determination of body surface area. (From Boothby, WM, and Sandiford, RB: Nomogram for determination of body surface area. N Engl J Med 185:227, 1921, with permission.)

TUBULAR REABSORPTION TESTS

Whereas measurement of the glomerular filtration rate is not a useful indication of early renal disease, the loss of tubular reabsorption capability is often the first function affected in renal disease. This is not surprising when one considers the complexity of the tubular reabsorption process.

Tests to determine the ability of the tubules to reabsorb the essential salts and water that have been nonselectively filtered by the glomerulus are collectively termed concentration tests. As mentioned previously, the ultrafiltrate that enters the tubules has a specific gravity of 1.010; therefore, after reabsorption one would expect the final urine product to be more concentrated. However, from our experience in performing routine urinalysis, we know that many specimens do not have a specific gravity higher than 1.010, yet no renal disease is present. This is because urine concentration is largely determined by the body's state of hydration, and the normal kidney will reabsorb only the amount of

water necessary to preserve an adequate supply of body water.

As can be seen in Figure 2–13, both specimens contain the same amount of solute; however, the urine density (specific gravity) of patient A will be higher. Therefore, control of fluid intake must be incorporated into laboratory tests that measure the concentrating ability of the kidney.

Throughout the years, various methods have been used to produce water deprivation, including the Fishberg and Mosenthal concentration tests that measured specific gravity. In the Fishberg test, patients were deprived of fluids for 24 hours prior to measuring specific gravity. The Mosenthal test compared the volume and specific gravity of day and night urine samples to evaluate concentrating ability. Neither test is in use today because the information provided by specific gravity measurements is most useful as a screening procedure and quantitative measurement of renal concentrating ability is best assessed through osmometry. However, persons with normal concentrating ability should have a specific gravity of 1.025 when deprived of fluids for 16 hours. Following overnight water deprivation a urine ***osmolarity*** of 800 mOsm or above indicates normal concentrating ability. Osmometry is particularly essential when evaluating neonates.[1,2]

Osmolarity

Specific gravity depends on the number of particles present in a solution and the density of these particles, whereas osmolarity is affected only by the number of particles present. When evaluating renal concentration ability, the substances of interest are small molecules, primarily sodium (molecular weight, 23) and chloride (molecular weight, 35.5). However, urea (molecular weight, 60), which is of no importance to this evaluation, will contribute more to the specific gravity than will the sodium and chloride molecules. Because all three molecules contribute equally to the osmolarity of the specimen, a more representative measure of renal concentrating ability can be obtained by measuring osmolarity.

An osmole is defined as 1 g molecular weight of a substance divided by the number of particles into which it dissociates. A nonionizing substance such as glucose (molecular weight, 180) contains 180 g per osmole, whereas sodium chloride (**NaCl**) (molecular weight, 58.5), if completely dissociated, contains 29.25 g per osmole. Just as we have the terms molality and molarity, we also have osmolality and osmolarity. An osmolal solution of glucose has 180 g of glucose dissolved in 1 kg of solvent, and an osmolar solution has 180 g of glucose dissolved in 1 L of solvent. In the clinical laboratory, the terms are used interchangeably, inasmuch as the difference under normal temperature conditions with water as the solvent is minimal. The unit of measure used in the clinical laboratory is the milliosmole (**mOsm**), because it is not practical when dealing with body fluids to use a measurement as large as the osmole (23 g of sodium per liter or kilogram).

The osmolarity of a solution can be determined by measuring a property that is mathematically related to the number of particles in the solution (colligative property) and comparing this value with the value obtained from the pure solvent. Solute dissolved in solvent causes the following changes in colligative properties: lower freezing point, higher boiling point, increased osmotic pressure, and lower vapor pressure.

Because water is the solvent in both urine and plasma, the number of particles present in a sample can be determined by comparing a colligative property value of the sample with that of pure water. Clinical laboratory instruments are available to measure freezing point depression and vapor pressure depression.

Freezing Point Osmometers

Measurement of freezing point depression was the first principle incorporated into clinical osmometers, and many

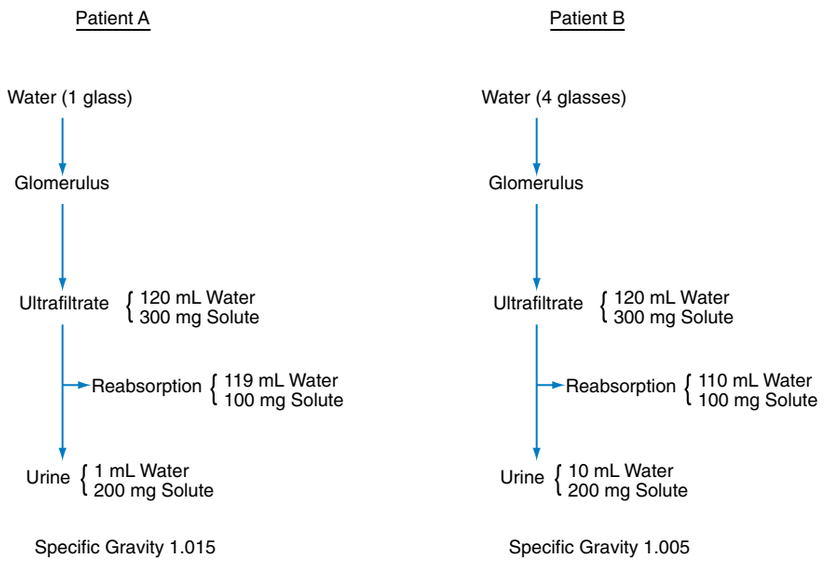


FIGURE 2–13 The effect of hydration on specific gravity.

instruments employing this technique are available. These osmometers determine the freezing point of a solution by supercooling a measured amount of sample to approximately −7°C. The supercooled sample is then vibrated to produce crystallization of water in the solution. The heat of fusion produced by the crystallizing water temporarily raises the temperature of the solution to its freezing point. A temperature-sensitive probe measures this temperature increase, which corresponds to the freezing point of the solution, and the information is converted into milliosmoles. Conversion is made possible by the fact that 1 mol (1000 mOsm) of a nonionizing substance dissolved in 1 kg of water is known to lower the freezing point 1.86°C. Therefore, by comparing the freezing point depression of an unknown solution to that of a known molal solution, the osmolarity of the unknown solution can be calculated. Clinical osmometers use solutions of known NaCl concentration as their reference standards because a solution of partially ionized substances is more representative of urine and plasma composition.

Vapor Pressure Osmometers

The other instrument used in clinical osmometry is called the vapor pressure osmometer; however, the actual measurement performed is the dew point (temperature at which water vapor condenses to a liquid). The depression of dew point temperature by solute parallels the decrease in vapor pressure, thereby providing a measure of this colligative property.

Samples are absorbed into small filter paper disks that are placed in a sealed chamber containing a temperature-sensitive thermocoupler. The sample evaporates in the chamber, forming a vapor. When the temperature in the chamber is lowered, water condenses in the chamber and on the thermocoupler. The heat of condensation produced raises the temperature of the thermocoupler to the dew point temperature. This dew point temperature is proportional to the vapor pressure from the evaporating sample. Temperatures are compared with those of the NaCl standards and converted into milliosmoles. The vapor pressure osmometer uses microsamples of less than 0.01 mL; therefore, care must be taken to prevent any evaporation of the sample prior to testing. Correlation studies have shown more variation with vapor pressure osmometers, stressing the necessity of careful technique.

Technical Factors

Factors to consider because of their influence on true osmolarity readings include lipemic serum and the presence of lactic acid or volatile substances, such as ethanol, in the specimen. In lipemic serum, the displacement of serum water by insoluble lipids produces erroneous results with both vapor pressure and freezing point osmometers. Falsely elevated values owing to the formation of lactic acid also will occur with both methods if serum samples are not separated or refrigerated within 20 minutes.[12] Vapor pressure osmometers will not detect the presence of volatile substances, inasmuch as they become part of the solvent phase; however, measurements performed on similar specimens using freezing point osmometers will be elevated. Comparisons of serum osmolarities run on cryoscopic and vapor pressure osmometers can be used as a method for rapid screening of comatose patients for alcohol ingestion.[8]

Clinical Significance

Major clinical uses of osmolarity include initially evaluating renal concentrating ability, monitoring the course of renal disease, monitoring fluid and electrolyte therapy, establishing the differential diagnosis of **hypernatremia** and **hyponatremia**, and evaluating the secretion of and renal response to ADH. These evaluations may require determination of serum in addition to urine osmolarity.

Normal serum osmolarity values are between 275 and 300 mOsm. Normal values for urine osmolarity are difficult to establish, because factors such as fluid intake and exercise can greatly influence the urine concentration. Values can range between 50 and 1400 mOsm.[15] Determining the ratio of urine to serum osmolarity can provide a more accurate evaluation. Under normal random conditions, the ratio of urine to serum osmolarity should be at least 1:1; after controlled fluid intake, it should reach 3:1.

The urine-to-serum osmolarity ratio, in conjunction with procedures such as controlled fluid intake and injection of ADH, is used to differentiate whether diabetes insipidus is caused by decreased ADH production or inability of the renal tubules to respond to ADH. Failure to achieve a urine to serum osmolarity ratio of 3:1 following injection of ADH indicates that the collecting duct does not have functional ADH receptors. In contrast, if concentration takes place following ADH injection, an inability to produce adequate ADH is indicated. Tests to measure the ADH concentration in plasma and urine directly are available for difficult diagnostic cases.[7]

Free Water Clearance

The urine-to-serum osmolarity ratio can be further expanded by performing the analyses using water deprivation and a timed urine specimen and calculating the **free water clearance**. The free water clearance is determined by first calculating the **osmolar clearance** using the standard clearance formula of

$$C_{osm} = \frac{U_{osm} \times V}{P_{osm}}$$

and then subtracting the osmolar clearance value from the urine volume in milliliters per minute.

EXAMPLE

Using a urine osmolarity of 600 mOsm (U), a urine volume of 2 mL/min (V), and a plasma osmolarity of 300 mOsm (P), calculate the free water clearance:

$$C_{osm} = \frac{600\ (U) \times 2\ (V)}{300\ (P)} = 4.0\ mL/min$$

$$C_{H_2O} = 2\ (V) - 4.0\ (C_{osm}) = -2.0$$

Calculation of the osmolar clearance tells how much water must be cleared each minute to produce a urine with the same osmolarity as the plasma. The ultrafiltrate contains the same osmolarity as the plasma; therefore, the osmotic differences found in the urine are the result of renal concentrating and diluting mechanisms. By comparing the osmolar clearance with the actual urine volume excreted per minute, it can be determined whether the water being excreted is more or less than the amount needed to maintain an osmolarity the same as that of the ultrafiltrate.

The above calculation shows a free water clearance of −2.0, indicating that less than the necessary amount of water is being excreted, indicating a possible state of dehydration. If the value had been 0, no renal concentration or dilution would be taking place; likewise, if the value had been +2.0, excess water would have been excreted, indicating decreased production of or response to ADH.[15] Therefore, calculation of the free water clearance can be used to determine the ability of the kidney to respond to the state of body hydration.

TUBULAR SECRETION AND RENAL BLOOD FLOW TESTS

Tests to measure tubular secretion of nonfiltered substances and renal blood flow are closely related in that total renal blood flow through the nephron must be measured by a substance that is secreted rather than filtered through the glomerulus. Impaired tubular secretory ability or inadequate presentation of the substance to the capillaries owing to decreased renal blood flow may cause an abnormal result. Therefore, an understanding of the principles and limitations of the tests and correlation with other clinical data are important in test interpretation.

The test most commonly associated with tubular secretion and renal blood flow is the *p*-aminohippuric acid (**PAH**) test. Historically excretion of the dye phenolsulfonphthalein (**PSP**) was used to evaluate these functions. Standardization and interpretation of PSP results are difficult, however, because of interference by medications and elevated waste products in patients' serum and the necessity to obtain several very accurately timed urine specimens. Therefore, the PSP test is not currently performed.

PAH Test

To measure the exact amount of blood flowing through the kidney, it is necessary to use a substance that is completely removed from the blood (plasma) each time it comes in contact with functional renal tissue. The principle is the same as in the clearance test for glomerular filtration. However, to ensure measurement of the blood flow through the entire nephron, the substance must be removed from the blood primarily in the peritubular capillaries rather than being removed when the blood reaches the glomerulus.

Although it has the disadvantage of being exogenous, the chemical PAH meets the criteria needed to measure renal blood flow. This nontoxic substance does not bind strongly to plasma proteins, which permits its complete removal as the blood passes through the peritubular capillaries. Except for a small amount of PAH contained in plasma that does not come in contact with functional renal tissue, all the plasma PAH is secreted by the proximal convoluted tubule. Therefore, the volume of plasma flowing through the kidneys determines the amount of PAH excreted in the urine. The standard clearance formula

$$C_{PAH}\,(mL/min) = \frac{U\ (mg/dL\ PAH) \times V\ (mL/min\ urine)}{P\ (mg/dL\ PAH)}$$

can be used to calculate the effective renal plasma flow. Based on normal hematocrit readings, normal values for the effective renal plasma flow range from 600 to 700 mL/min, making the average renal blood flow about 1200 mL/min. The actual measurement is renal plasma flow rather than renal blood flow, because the PAH is contained only in the plasma portion of the blood. Also, the term "effective" is included because approximately 8 percent of the renal blood flow does not come into contact with the functional renal tissue.[9]

The amount of PAH infused must be carefully monitored to ensure accurate results; therefore, the test is usually performed by specialized renal laboratories. Nuclear medicine procedures using radioactive hippurate can determine renal blood flow by measuring the plasma disappearance of a single radioactive injection and at the same time provide visualization of the blood flowing through the kidneys.[4,17]

Titratable Acidity and Urinary Ammonia

As discussed previously, the ability of the kidney to produce an acid urine depends on the tubular secretion of hydrogen ions and production and secretion of ammonia by the cells of the distal convoluted tubule. A normal person excretes approximately 70 mEq/day of acid in the form of either titratable acid ($\mathbf{H^+}$) or ammonium ions ($\mathbf{NH_4^+}$). In normal persons, a diurnal variation in urine acidity consisting of alkaline tides appears shortly after arising and postprandially at approximately 2 PM and 8 PM. The lowest pH is found at night.[10]

The inability to produce an acid urine in the presence of metabolic acidosis is called renal tubular acidosis. This condition may result from impaired tubular secretion of hydrogen ions associated with the proximal convoluted tubule or defects in ammonia secretion associated with the distal convoluted tubule.

Measurement of urine pH, ***titratable acidity***, and urinary ammonia can be used to determine the defective function. The tests can be run simultaneously on either fresh or toluene-preserved urine specimens collected at 2-hour intervals from patients who have been primed with an acid load consisting of oral ammonium chloride. By titrating the amount of free H^+ (titratable acidity) and then the total acidity of the specimen, the ammonium concentration can be calculated as the difference between the titratable acidity and the total acidity.[5]

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STUDY QUESTIONS

1. State two major processes associated with the peritubular capillaries.

2. Why is the glomerulus referred to as a nonselective filter?

3. How do the capillary endothelial cells in the glomerulus differ from other capillary endothelial cells?

4. When is the kidney stimulated to produce renin? What is the primary chemical affected by the renin-angiotensin-aldosterone system?

5. Define the term ultrafiltrate of plasma.

6. List four substances reabsorbed by active transport and two substances reabsorbed by passive transport.

7. When will a substance that is usually completely reabsorbed by active transport appear in the urine?

8. How does reabsorption in the descending loop of Henle differ from reabsorption in the ascending loop? What is the primary reason for this difference?

9. Why is water not always reabsorbed from the collecting duct when it passes through the medulla?

10. When the body is dehydrated, is the production of ADH increased or decreased?

11. If a waste product is not filtered at the glomerulus, how can it be removed from the blood?

12. Name a chemical that is filtered by the glomerulus and reabsorbed and secreted by the tubules.

13. How does tubular secretion maintain the buffering capacity of the blood?

14. How will failure of the distal convoluted tubule to produce ammonia affect urine pH?

15. State whether the following are endogenous or exogenous test substances: urea, inulin, creatinine, beta$_2$ microglobulin, and radionucleotides.

16. How will bacterial breakdown of urine creatinine, increased plasma chromogens, and an incomplete urine collection affect the results of a creatinine clearance test?

17. Given the following information, calculate the creatinine clearance: 24-hour urine volume = 720 mL, plasma creatinine = 1.5 mg/dL, and urine creatinine = 300 mg/dL.

18. When calculating creatinine clearance tests performed on children, are any additional calculations required? Why or why not?

19. Why is the creatinine clearance test not useful for detecting early renal disease?

20. Why do tests for renal concentrating ability incorporate water deprivation?

21. Why is urine osmolarity more representative of urine concentration than specific gravity?

22. State the two colligative properties measured by clinical osmometers.

23. With which clinical osmometer is specimen evaporation of greatest concern?

24. A random urine sample has a urine osmolarity of 305 mOsm and a serum omolarity of 295 mOsm. Is this normal or abnormal?

25. Given the following information, calculate the free water clearance and interpret the result: Urine volume = 720 mL in 6 hours, urine osmolarity = 75 mOsm, and plasma osmolarity = 300 mOsm.

26. Explain the relationship of tubular secretion to tests for renal blood flow.

27. Given the following information, calculate the effective renal plasma flow: Urine volume = 240 mL in 2 hours, urine PAH = 150 mg/dL, and plasma PAH = 0.5 mg/dL.

28. Why is the result obtained in Study Question #27 called effective renal plasma flow rather than renal blood flow?

29. Define the term renal tubular acidosis.

30. Following administration of ammonium chloride to a normal person, will the levels of titratable acidity and urinary ammonia be decreased or increased?

CASE STUDIES AND CLINICAL SITUATIONS

1. A 44-year-old man diagnosed with acute tubular necrosis has a blood urea nitrogen of 60 mg/dL and a blood glucose level of 100 mg/dL. Urinalysis results are as follows:

COLOR: Dark yellow	KETONES: Negative
CLARITY: Hazy	BLOOD: Moderate
SPECIFIC GRAVITY: 1.020	BILIRUBIN: Negative
pH: 6.0	UROBILINOGEN: Normal
PROTEIN: 2+	NITRITE: Negative
GLUCOSE: 2+	LEUKOCYTE ESTERASE: Trace

a. State the renal threshold for glucose.
b. What is the significance of the positive urine glucose and normal blood glucose?
c. Considering the diagnosis, what specific gravity result would be expected?
d. What could be causing this discrepancy?
e. How could a more representative measure of urine concentration be obtained?

2. A patient develops a sudden drop in blood pressure.
a. Diagram the reactions that take place to ensure adequate blood pressure within the nephrons.
b. How do these reactions increase blood volume?
c. When blood pressure returns to normal, how does the kidney respond?

3. A physician would like to prescribe a nephrotoxic antibiotic for a 60-year-old man with a temperature of 102°F. The patient has a serum creatinine level of 1.0 mg/dL.
a. How can the physician determine whether it is safe to prescribe this medication before the patient leaves the office?
b. Can the medication be prescribed to this patient with a reasonable assurance of safety?

4. A laboratory is obtaining erratic serum osmolarity results on a patient who is being monitored at 6 AM, 12 PM, 6 PM, and 12 AM. Osmolarities are not performed on the night shift; therefore, the midnight specimen is run at the same time as the 6 AM specimen.
a. What two reasons could account for these discrepancies?
b. If the laboratory is using a freezing point osmometer, would these discrepancies still be encountered? Why or why not?
c. If a friend was secretly bringing the patient a pint of whiskey every night, would this affect the results? Explain your answer.

5. Following overnight (6 PM to 8 AM) fluid deprivation, the urine-to-serum osmolarity ratio in a patient who is exhibiting polyuria and polydipsia is 1:1. The ratio remains the same when a second specimen is tested at 10 AM. Vasopressin is then administered subcutaneously to the patient, and the fluid deprivation is continued until 2 PM when another specimen is tested.
a. What disorder do these symptoms and initial laboratory results indicate?
b. If the urine to serum osmolarity ratio on the 2 PM specimen is 3:1, what is the underlying cause of the patient's disorder?
c. If the urine to serum osmolarity ratio on the 2 PM specimen remains 1:1, what is the underlying cause of the patient's disorder?