EXCRETION

Introduction

Humans must get rid of two types of wastes. Wastes from the digestive system (feces) and wastes from metabolic activities (sweat & urine). Removing digestive wastes (pooping) is called egestion. Removing metabolic wastes is called excretion.

Major Metabolic Wastes

Table (1) summarizes the four types of metabolic wastes produced by humans (and other animals), and the type of chemical reactions that produce them.

METABOLIC WASTE	TYPE OF CHEMICAL REACTIONS
Water	Dehydration synthesis & respiration
Carbon dioxide	Cellular respiration
Salts	Neutralization
Urea	Protein metabolism, deamination

Table 1. The major metabolic waste products of human.

Dehydration synthesis = reactions in which small molecules are combined to form large molecules

Cellular respiration = chemical reaction that releases energy from organic molecules (usually glucose and fatty acids)

Neutralization = reactions between acids & bases

Deamination = removal of amino groups from protein molecules

Remarks on the nitrogenous wastes

Amino acids

Digestion of proteins leads to hydrolyze them to their respective amino acids. Excess of amino acids are excreted as such without any change by certain animals such as molluscs, echinoderma etc. Human excrete minor amounts of some amino acids in the urine.

Ammonia

Ammonia is formed as a result of deamination of amino acids. Ammonia is a poisonous, so it presents only in low concentration in the blood of many animals. In humans, levels of only 5 mg per 100 ml of blood are toxic, and the normal concentration of this metabolite is only 1 to 3 micrograms per 100 ml of human blood. Fishes do not have concentrations of ammonia greater than 0.1 mg (100 micrograms) per 100 ml of blood. Because ammonia is toxic, it apparently combined with glutamic acid to give glutamine. Glutamine is carried through the blood circulation to a membrane next to the surrounding water (the gills for fishes and certain other animals) where it converted again to glutamic and ammonia, which is excreted into the water (Fig. 1). Humans excrete ammonia in

the urine in the form of ammonium ions (NH_4^+), and presumably the ammonia is formed also from glutamine in the kidneys. The amount of ammonium ions excreted is not very large, and there is no detectable excretion of ammonia by normal humans. The reversible reaction between ammonia and glutamic acid can be described as follows:
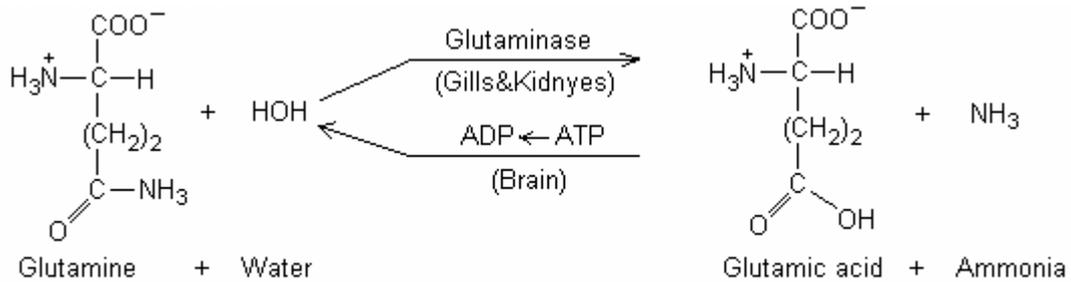

Fig. 1. Formation of ammonia from glutamine in either kidneys of mammals or gills of fishes.

Urea

It is the major nitrogenous waste product derived from amino acid metabolism. It is also excreted by some animal as an end product of purine bases metabolism. Urea is less toxic and more soluble in water than ammonia. Levels between 18 and 38 mg/100 ml of blood are considered normal in humans. In sea sharks, concentrations of urea as much as 2 g/100 ml of blood are found, while in freshwater sharks concentration of urea was much less, it is about 0.6 g/100 ml of blood. This gives evidence that urea is involved in regulation of the osmotic pressure in salty sea water sharks. In livers of mammals, urea is synthesized from ammonia through a series of reactions known as urea cycle (Fig. 2). Ammonia is firstly converted to the carbamyl phosphate, and an enzyme called arginase is required to release of urea from arginine in the presence of. Energy is required for synthesis of urea.
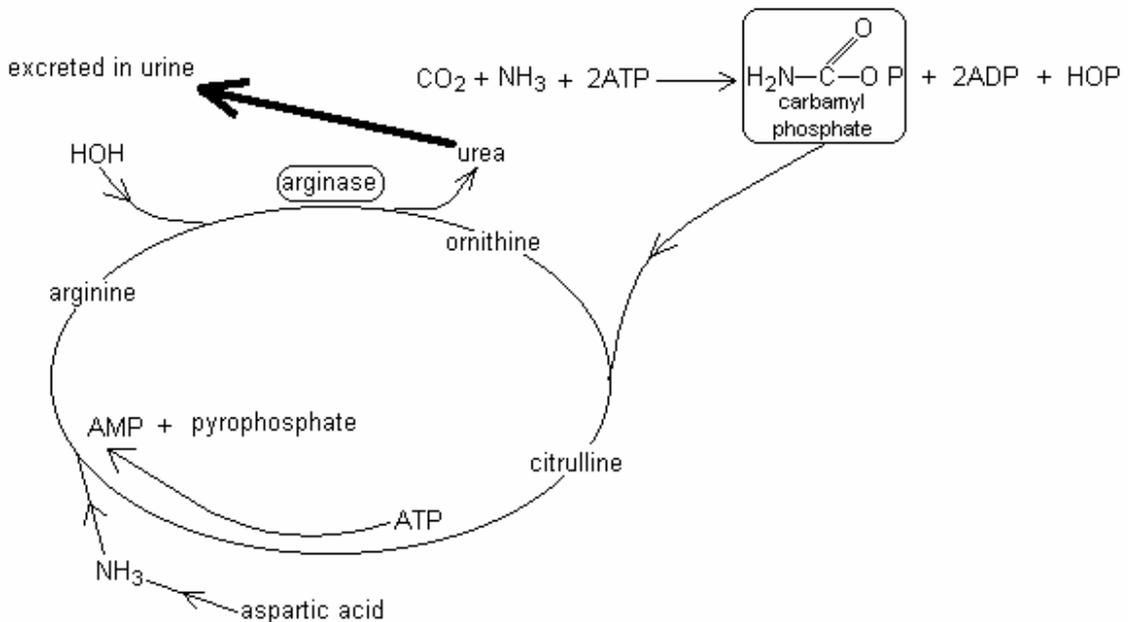

Fig. 2. Urea cycle in mammals. Note that, CO_2 and NH_3 are added to the reaction in the form of carbamyl phosphate.

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Uric acid

It excreted by the animals which conserve water, at least during parts of their life cycle, such as birds, terrestrial reptiles, insects, and some snails. In this case ammonia is converted into uric acid. It is less toxic than ammonia but it is insoluble in water. Uric acid is excreted in crystalline form mixed with, but not dissolved in, small amounts of water. In fowls (chickens and turkeys), blood level of uric acid is about 5 mg/100 ml, while insects have as much as 20 mg uric acid/100 ml of blood. The synthesis of uric acid is a complex, energy-requiring process. Humans excrete uric acid as an end product of the metabolism of purine bases adenine and guanine. Uric acid is formed also by a direct synthesis from 5-phosphoribosyl pyrophosphate and glutamine. Levels of uric acid in human blood is about 4.5 mg/100 ml in men and 3.5 mg/100 ml in women.

Trimethylamine oxide

This nitrogenous excretory product is formed in marine teleost fishes.

Guanine

This nitrogenous excretory product is formed in the spiders and swine. Its solubility is very low.

Allantoin

It is formed from uric acid via an oxidation reaction catalyzed by an enzyme uricase. It excreted in some mammals, reptiles, and molluscs.

Hippuric acid

This acid is found in mammals. The benzoic acid present in the food of mammals is removed and it combines with glycine to form hippuric acid.

Ornithuric acid

In birds, this excretory product is formed by combination of the nitrogenous compound ornithine with benzoic acid which is present in the food of these animals.

Creatine

Creatine is synthesized in the liver from three amino acids namely, arginine, glycine, and methionine. Creatine is liberated into the blood and is taken up by the muscle when required. In skeletal muscle, it is phosphorylated to form creatine phosphate, which is an important energy store for ATP synthesis. The excess of creatine is excreted along with urine.

Creatinine

It is formed in the body from creatine phosphate. Creatine is not converted directly to creatinine. The rate of creatinine excretion is constant from day to day.

Classification of animals on basis of types of nitrogenous compounds excreted

Ammonotelic

The animals of this group excrete nitrogen mainly in the form of ammonia. This is predominantly among aquatic animals, e.g. certain unicellular animals, teleosts, tadpoles, polycheate annelids, Aplysia, molluscs, crocodiles, and crustaceans.

Ureotelic

In these animals, nitrogen excreted predominantly in the form of urea, e.g. mammals, amphibians, and elasmobranchs. Among fishes, both ammonotelic and ureotelic metabolism are present.

Uricotelic

Animals are described as being uricotelic when nitrogen is excreted predominantly in the form of uric acid, e.g. birds, insects, lizards, snakes, and some gastropods.

HUMAN EXCRETORY ORGANS

There are 4 excretory organs in human: The skin, the lungs, the liver, and the kidney (Urinary system).

THE SKIN

The skin excretes the sweat outside the body through numerous pores in the surface of this organ. Sweat is a mixture of three metabolic wastes: water, salts, and urea. So as you sweat, your body accomplishes two things: 1) sweating has a cooling effect on the body, and 2) metabolic wastes are excreted.
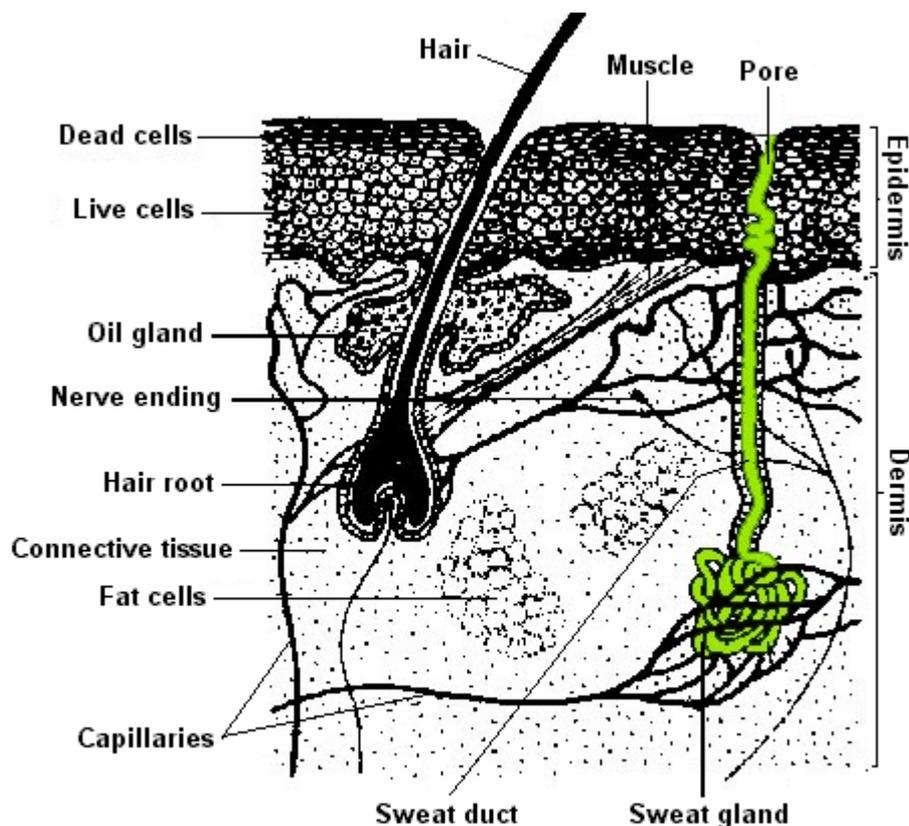

Figure 3. Localization of the sweat gland in the skin

The skin is formed of two layers; the thin epidermis at the top, and the thicker dermis below. The inner layer of skin (dermis) contains the oil glands, hair follicles, fatty layers, nerves, and sweat glands. The sweat gland leads to the sweat duct (tube) which opens on the skin surface through a pore (Fig. 3).

Sweat formation

Notice that the sweat gland is a tubular structure tangled with the blood capillaries. This close association of tubes allows wastes (namely water, salts, and urea) to diffuse from the blood into the sweat gland. When body temperature rises, the fluid (sweat) is released from the gland, travels through the duct, and reaches the skin surface through openings called pores.

THE LUNGS

Cellular respiration occurs in every living cell in your body. It is the reaction that provides energy (in the form of ATP molecules) for cellular activities. If respiration stops, the cell no longer has energy for cellular activities, and the cell dies. As respiration occurs carbon dioxide is produced as a waste product. As the carbon dioxide accumulates in body cells, it eventually diffuses out of the cells and into the bloodstream, which eventually circulates to the lungs. In the alveoli of the lungs, carbon dioxide diffuses from the blood, into the lung tissue, and then leaves the body every time we exhale. Some water vapor also exits the body during exhalation.

THE LIVER

The liver is a large, important organ in our bodies. Its numerous functions make it "part" of the circulatory, digestive, and excretory systems. Liver as an excretory organ acts to breakdown some proteins and other nitrogenous compounds by a process called deamination. As a result of these reactions, a nitrogenous waste called *urea* is formed. Liver as well as helps in excreting toxic substances, drugs, and their derivatives; and bile pigments and cholesterol.

THE URINARY SYSTEM IN HUMAN (THE KIDNEY)

Generally, excretion means the separation and elimination of waste materials from the body through a special structure called the excretory organ. Specifically, the act of excretion is a two-fold function: it eliminates substances that have reached their threshold concentration in the blood, and it retains or reabsorbs substances that are below this concentration. Both functions, however, are homeostatic, tending to maintain a stable environment within the body. The major functions of the excretory systems can be summarizes as follows:

1. Maintenance of proper concentrations of individual ions (Na^+, K^+, Cl^-, H^+, etc.).
2. Maintenance of proper body volume by regulating water content.
3. Maintenance of osmotic concentrations, which result from the ability of the excretory systems to control water and electrolytes contents in the body.
4. Removal of metabolic end products (e.g., urea, uric acid, etc.).
5- Removal of foreign substances and/or their metabolic products.

EXTERNAL STRUCTURE

Two Kidneys

They are dark, red, bean-shaped and lie in the upper part of the abdominal cavity against the dorsal body wall (Fig. 4). They are embedded in a protective layer of fat and connective tissue. The right kidney is slightly on a lower level than the left. Each Kidney is about 4½ inches long, 2½ inches broad, and over one inch thick. The weight of each kidney in adult human is about 150 g, so they represent about 0.5% of the total weight of the body.

Two Ureters

They are two slender muscular tubes which take their origin at the hilum of each kidney (from the renal pelvis) and run down to join the urinary bladder.

The Urinary Bladder

The bladder has an elastic wall and placed in the lower part of the abdominal cavity. It supplied with a sphincter muscles at its connection with both the ureters and urethra.

The Urethra

It is a muscular tube which carried the urine from the bladder to the outside

Figure 4. The human excretory system. The left kidney has been opened to show the internal structure.

Renal Vein and Artery

Each kidney receive a renal artery from the aorta, which brings the blood into the kidney. From each kidney, a renal vein is extended to the inferior vena cava, which carries the blood back to the heart.
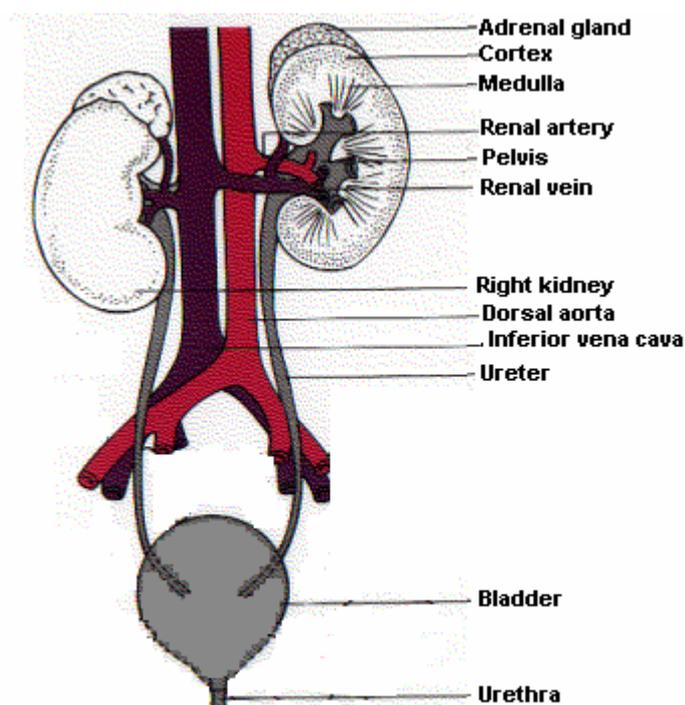
THE KIDNEY

INTERNAL STRUCTURE

Examination of vertical section of the kidney (Fig. 5) shows that the kidney is made up of:

An Outer Cortex

It made of a dark red tissue, due to the presence of all glomeruli which contain tufts of blood capillaries. The cortex contains all the proximal tubules and distal tubules, and cortical collecting ducts.

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An Inner Medulla

It is made of lighter tissue, due to its relative low blood supply. Medulla has a radial appearance due to the presence of loops of Henle, the vasa recta, and medullary collecting tubules. It is subdivided into: (i) an outer medulla, which lies next to the cortex and (ii) an inner medulla which extends out into the renal sinus forming renal papillae. Medulla is differentiated to form a number of cone-like structure known as renal pyramids (10-15) with their apical ends projecting as renal papillae into the calyces of the pelvis.

The Pelvis

It is a funnel-shaped structure which has at its free end number of cup-like cavities called calyces (*sing. calyx).* The pelvis leads to the ureter.
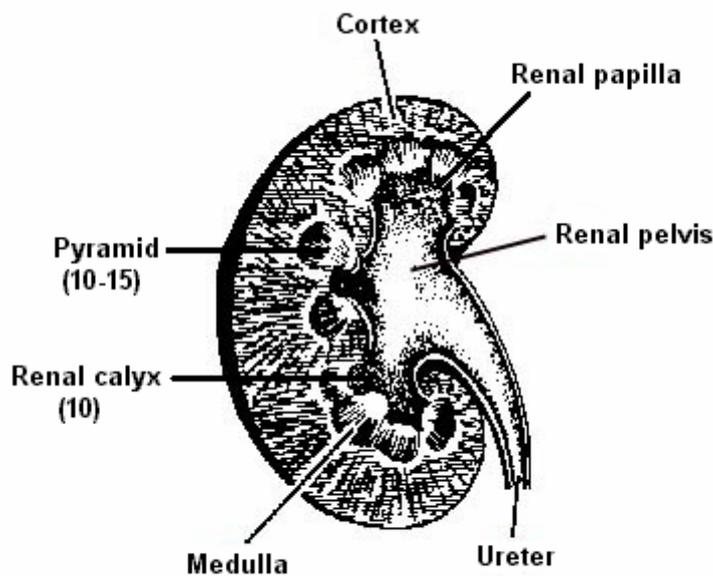

Figure 5. Sagittal section in the kidney showing the internal structure.

PHYSIOLOGICAL FUNCTIONS OF KIDNEYS

Excretion of waste products

Kidneys excrete the waste products which contains nitrogen and sulphur, as well as ketone bodies. They aid in excretion of the drugs, toxic substances, and their derivatives, e.g. penicillin.

Maintenance of constant volume and composition of inside the body

The kidneys maintain constant volume of body fluids, osmotic pressure, and blood pressure, hence they protect the body from diseases, by excreting excess water and electrolytes. For instances, (i) excess intake of sodium salts (NaCl) leads to hypernatermia accompanied with increased water retention which lead to increase in blood pressure and osmotic pressure, as well as development of oedema. As kidneys capable of to remove the excess NaCl, they prevent the mentioned syndromes. (ii) excess intake of potassium (K^+) produces hyperkalemia which leads to weakness of muscles and this causes troubles in the heart and abdominal muscles. Kidneys remove the excess K^+ and keep normal muscle function, e.g. heart beats.

Regulation of arterial blood pressure (ABP)

In case of hypoxia due to hypotension, the kidneys secrete the enzyme renin, via the juxtaglomerular tissue, which converts plasma angiotensigogen to angiotensin I that converted by convertase into angiotensin II. Angiotensin II acts as (i) a vasoconstrictor leading to raising the blood pressure and (ii) a stimulator for the secretion of both aldosterone from the adrenal cortex and antidiuretic hormone from the posterior pituitary, which cause Na^+ and water retention hereby increasing blood volume and then restoring the blood pressure.

The kidneys synthesize a number of prostaglandins (PGs), a vasoactive substances, that act to increase the ABP. PGE_2 acts as a vasodilator which can modulate the vasoconstriction induced by sympathetic stimulation.

Regulation of blood pH through preserving acid-base balance

In case of acidosis, they secrete H^+ and react it with ammonia (NH_3) forming ammonium (NH_4), which excreted as NH_4 salts in the urine. While, in case of alkalosis, the kidneys decrease the secretion of H^+, synthesis of NH_4, and reabsorption of bicarbonate ($HCO3^-$); and they increases reabsorption of Cl^-.

Enzyme formation

The kidneys synthesize enzymes such as histaminase to destroy the histamine, phosphatase to remove inorganic phosphate from organic compounds, and cholinesterase to destroy acetylcholine.

Endocrine function

They regulate the conversion of vitamin D to 1,25 dihyroxycholecalciferol (and also 24,25 dihyroxcholecalciferol) which facilitate the intestinal absorption of calcium and phosphate. It also acts on bone by mobilizing the calcium ion. PTH is required for renal synthesis of 1,25 dihyroxcholecalciferol.

Detoxification

In the kidney, the toxic substance is converted to a non-toxic compound. For example, the kidneys convert benzoic acid to the hippuric acid by combination with glycine and excrete it through urine to outside. This process occurs mainly in the liver.

THE NEPHRON

The substance of the kidney is made up of a number of structural and functional units called nephrons (Fig. 6). Each human kidney contains one million nephrons or more. The nephrons are concerned with the separation of urine from the blood.

It should be noted that there are 2 basic types of nephrons:

Cortical nephrons: They represent 85% of the nephrons in the kidney. Except for a small portion of the loop of Henle, they're entirely located within the renal cortex. They will play a large role in making sure the blood has the correct ionic and chemical make-up.

Juxtamedullary nephrons: Their renal corpuscles are located very close to the cortex-medulla junction. Their loops of Henle extend deep into the medulla and can be quite long. They play an important role in the body's ability to concentrate urine, i.e. they are very involved in water reabsorption.

Structure of the Nephron
I- Malpighian corpuscle.
II- Coiled uriniferous tubule (Proximal tubule, loops of Henle, and distal tubule).

I- Malpighian corpuscle

Malpighian corpuscles are placed in the cortex and each one is composed of Bowman's capsule enclosing inside its cavity a network of blood capillaries known as glomerulus.

Bowman's capsule

The Bowman's capsule is composed of a double walled sac, enclosing inside its cavity a network of blood capillaries, the glomerulus. The capsule is formed of two layers (Fig. 7):

(1) An internal or visceral layer which is formed from epithelial cells that rests on a dense basement membrane. The latter membrane is the only intact barrier between blood inside the capillaries and the filtrate in Bowman's capsule. Epithelial cells do not provide a continuous covering for the basement membrane and capillaries, being attached to them only by means of processes (pedicles or feet, Fig. 8), so they called podocytes. The visceral layer is highly involved in filtrate formation.


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1 = Renal corpuscle (Bowman's
    capsule and glomerulus)
2 = Proximal convoluted tubule
3 = Proximal straight tubule
4 = Descending thin limb of Henle
5 = Ascending thin limb of Henle
6 = Ascending thick limb of Henle
    (Distal straight tubule)
7 = Macula densa
8 = Distal convoluted tubule
9 = Cortical collecting duct
10 = Outer medullary collecting duct
11 = Inner medullary collecting duct
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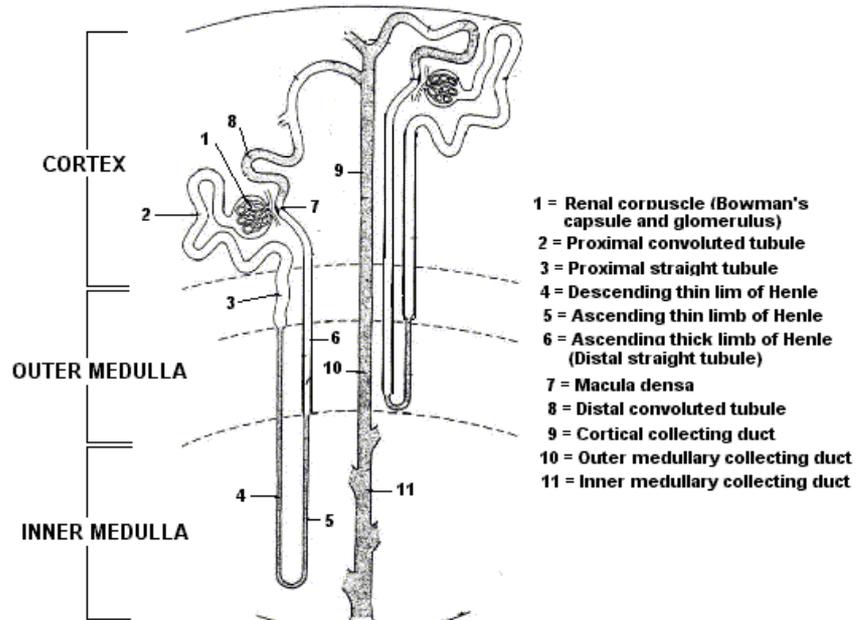
Figure 6. Scheme of nephron. This schem shows a short-looped and a long-looped nephron together with the collecting system.
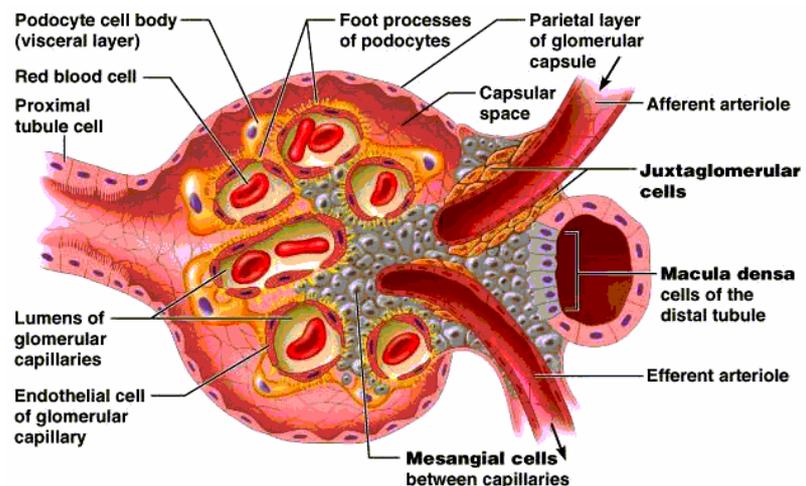

Figure 7. Scheme of the section of the glomerulus along with juxtaglomerular apparatus.

(2) An external or parietal layer of Bowman's capsule which is formed of simple squamous epithelium surrounded by reticular fibers. It plays no role in filtrate

formation. Between these two layers, there is a space known as capsular space which receives the fluid filtered from the capillaries.

Glomerulus

The Bowman's capsule receives a small arteriole, afferent arteriole, which branches into a tuft of capillary loops about 50 in number, the glomerulus, within the capsule (Fig. 7). The capillaries then reunite and form another small arteriole, the efferent arteriole, which leaves the capsule. The afferent arteriole is wider than the efferent one, and as a result, a pressure builds

Figure 8. The podocyte.

up within the capillaries which causes filtration of the water and dissolved substances out of the capillaries and into the capsule. The average diameter of glomerulus is about 200µm and the total surface of glomerular capillaries in the two human kidneys is estimated to be about 1.5 m^2. The glomeruli are found only in the cortex.
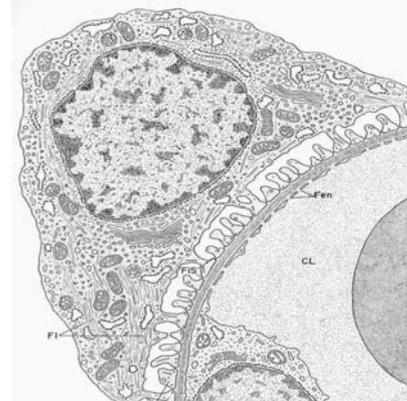
Filtration membrane

The filtration membrane which separates the blood from the glomerular filtrate in Bowman's capsule consists of 3 layers (Fig. 9).

(1) The capillary endothelium: It is a single layer, fenestrated with pores that are 70-90 nm in diameter. It prevents the blood cells and platelets from coming into contact with the basement membrane.

(2) Bowman's capsular epithelium: It lies on the top of the glomerular capillaries. Epithelial cells do not provide a continuous covering for the basement membrane and capillaries, being attached to them only by means of processes (pedicles or feet), so they called podocytes. So, the feet of these cells interdigitate to form filtration slits along with the capillary wall. The slits are approximately 25 nm wide, and each is closed by a thin membrane

(3) The basal lamina or the basement membrane: It is located between the 2 cellular layers mentioned previously. It is a meshwork hereby it acts as a sieve or
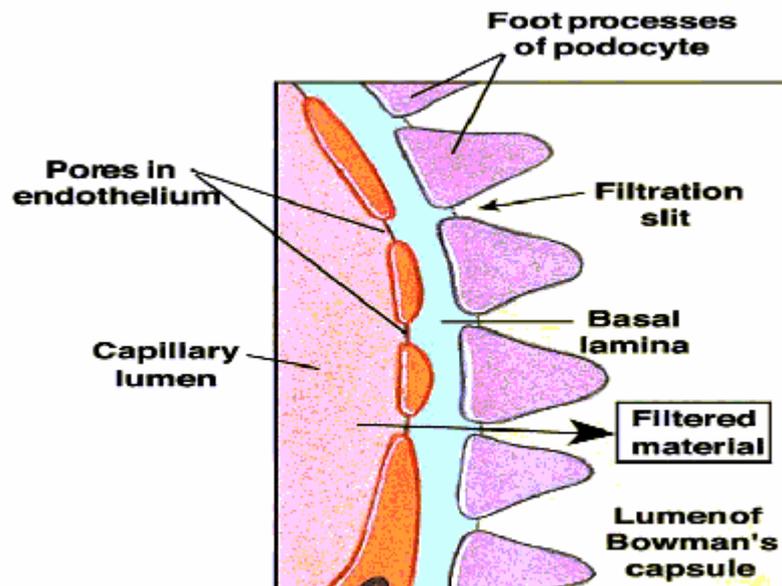

Figure 9. The filtration membrane.

barrier which prevents the filtration of large molecules, e.g. proteins as albumin or larger than it.

Mesangial cells

Mesangial cells are stellate cells which located between the basal lamina and the endothelium (Fig. 7). The cells are especially common between 2 neighboring capillaries, and in these locations the basal membrane forms a sheath shared by both capillaries. The mesangial cells are contractile and play a role in the regulation of glomerular filtration. Also, they secrete various substances, take up and catabolize immune complexes by phagocytosis, and are involved in the production of glomerular disease.

Juxtaglomerular apparatus (JGA)

JGA is composed of 3 types of cells (Fig. 10).

(1) Juxtaglomerular cells: They are located in the wall of the afferent arteriole, before its entering the Bowman's capsule. The cells synthesize, store, and release a proteolytic enzyme known as renin. The secretion of this enzyme is stimulated by Na^+ concentration and hypotension of the blood, so these cells act as mechanoreceptors, sensing blood pressure in the arterioles. Juxtaglomerular cells also secrete the renal erythropoietic factor that stimulates erythropoiesis.

(2) Macula densa cells: These tubular epithelial cells are located in the end portion of the thick ascending limb of Henle loop, i.e. at the starting part of distal tubule, where it passes close to afferent arteriole and efferent arteriole. They differ histologically from the other tubular cells forming macula densa, which are stimulated by decreased Na^+ concentration in the tubular fluid.

(3) Lacis cells: They are group of cells located between the juxtaglomerular cells and macula densa. Lacis cells function as a link between the macula densa and the glomerular arterioles.

Summary of the functions of JGA

1- Release of renin which regulate the arterial blood pressure: In case of hypoxia (ischaemia of the kidney), the kidneys secrete the enzyme renin, by juxtaglomerular cells, which converts plasma

Figure 10 . Juxtaglomerular cell.

angiotensigogen (α_2-globulin) to angiotensin I, which is inactive. Angiotensin I is converted by plasma enzyme of convertase into angiotensin II. Angiotensin II acts as (i) a vasoconstrictor leading to raising the blood pressure. (ii) a stimulator for the secretion of both aldosterone from the adrenal cortex, which cause Na^+ retention and antidiuretic hormone from the posterior pituitary, which cause water retention thereby increasing blood volume and restoring the blood pressure. (iii) Direct stimulator on the proximal convoluted tubule to enhance Na^+ and water reabsorption. (iv) Stimulator for thirst sensation.

2- Juxtaglomerular cells secrete the erythropoietin hormone which stimulates erythropoiesis (development of red blood cell from the bone marrow).
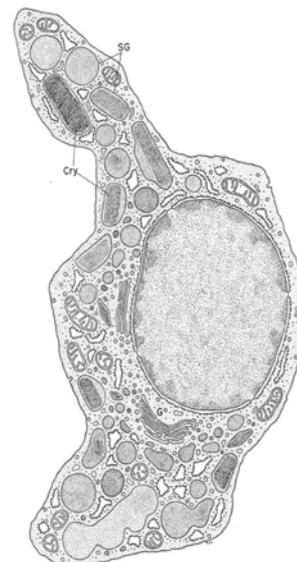
II- Coiled uriniferous tubule
(Proximal tubule, loop of Henle, and distal tubule)

The coiled uriniferous tubule consists of three distinct parts which are differed histologically in relation with differences in function, the proximal tubule, the loop of Henle, and the distal tubule (Fig. 6).

The proximal convoluted tubule

It begins from the capsular space and descends toward the medulla and thins out to form the loop of Henle. Its length is about 15 mm and diameter about 55μm. The proximal convoluted tubule are made up of highly differentiated active transport cells (Fig. 11). It possesses a brush borders which forms the micovilli that serve to increase greatly the surface available for absorption. Basal infoldings of the plasma membrane partition the cytoplasm into narrow compartments, perpendicular to the base of

Figure 11. The proximal tubule cubiodal cell.

the cell. These compartments are filled up with mitochondria arranged end to end in a single file. The mitochondria provide the ATP that power much of the reabsorption and secretion. Between the bases of the cells, there are extensions of the extracellular space called the lateral intercellular spaces.
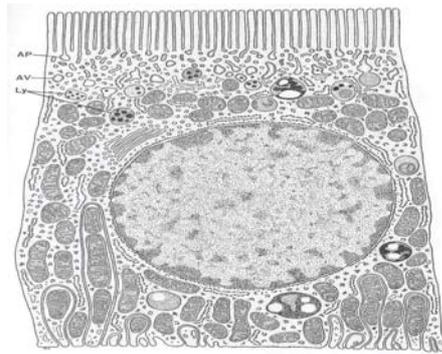
The loops of Henle

It commences with a descending limb which dips down into the medulla. As it does so, the cuboidal cells are replaced by squamous flat type of epithelial cells, which are permeable to water and sodium (Fig. 12). The loop of Henle dips only for a short distance in medulla when the glomerulus lies near the surface of the cortex (cortical nephrons), whereas if the glomerulus is situated near the corticomeduulary junction, the loop of Henle dips for a greater distance in pyramids (juxtamedullary nephrons). In human, only 15% of the nephrons have long loops. The total length of the thin segment of the loop varies from 2 to 14 mm in length. The loop of Henle ends in the thick segment of the ascending limb, which

Figure 12 . Squamous epithelial cell of thin descending limb of Henle's loop.

is about 12 mm in length. The cells in the thick segment are cuboid, impermeable to water, and have numerous mitochondria, and the basilar portion of their cell membranes are extensively invaginated. The thick segment of loop of Henle reaches the glomerulus of the nephron and passes close to its afferent arteriole and efferent arteriole forming a special cells called macula densa, a part from JGA.

Distal convoluted tubule

It is about 5 mm long. It starts from the macula densa. In comparison with the epithelium of the proximal convoluted tubule, its cuboidal epithelium possesses no micovilli (Fig. 13). However, the basal parts exhibit infoldings of the plasma membrane partitioning the cytoplasm into narrow compartments perpendicular to the base of the cells. Each compartment is filled up with abundance of mitochondria. These features suggest that these cells are more involved in the active secretion of solutes. As the distal segment approaches the collecting tubule, it undergoes some cytological differentiation, i.e. appearance of isolated, large, granular cells (intercalated cells).
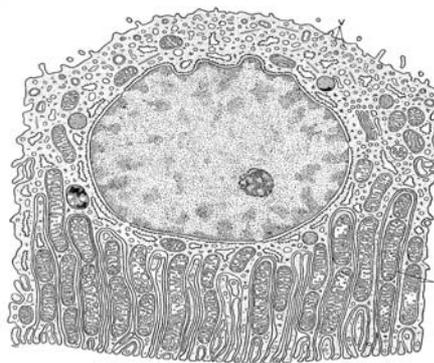

Figure 13. The distal tubule cuboidal epithelial cell.

The Collecting Tubules and Papillary Ducts

Because of the separate embryonic origin these are not included within the term nephron. The epithelium of the collecting ducts is made up of principal cells (P cells, Fig. 14), which are predominant, and intercalated cells (I cells), which are present in smaller numbers. P cells are columnar, lack microvilli, and have spherical nuclei and few granules. Their cytoplasm is relatively free of mitochondria and other protoplasmic inclusions. They are involved in Na^+ reabsorption and vasopressin-stimulated passive water reabsorption. The I cells, which are also found in the distal tubules, have more microvelli, cytoplasmic vesicles, and mitochondria. They are concerned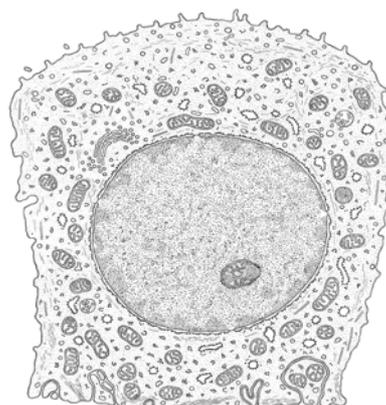 with acid secretion and HCO^- transport. Intercommunications of collecting tubules proceed in the cortex and medulla until the inner zone of the pyramid. Several collecting tubules fuse to form one papillary duct draining into a minor calyx.

Figure 14. The principal cell of the collecting duct.

The Interstitial Space

There is no interstitial space in the cortex, i.e. the tubules and peritubular venous capillaries being contiguous. In medulla, there is interstitial space, as the parallel tubes are separated by connective tissue, which is thicker towards the apex of the pyramids. Some of the cells in the interstitial tissue have a secretory function and called type I medullary interstitial cells. These cells contain lipid droplets and probably secrete prostaglandins, predominantly PGE_2.

Lymphatic vessels

The kidney has an abundant lymphatic supply that drains via the thoracic duct into the venous circulation in the thorax. Lymphatic system is present in cortical substance, but its role in the kidney is obscure.

RENAL BLOOD SUPPLY

A short wide renal artery is branched from the abdominal aorta, one for each kidney, and enters the hilum of the kidney where it breaks up into numerous branches, the interlobular arteries, which pass outward between the renal pyramids to the junction of the cortex with the medulla (Figures 15 and 16). At the corticomedullary 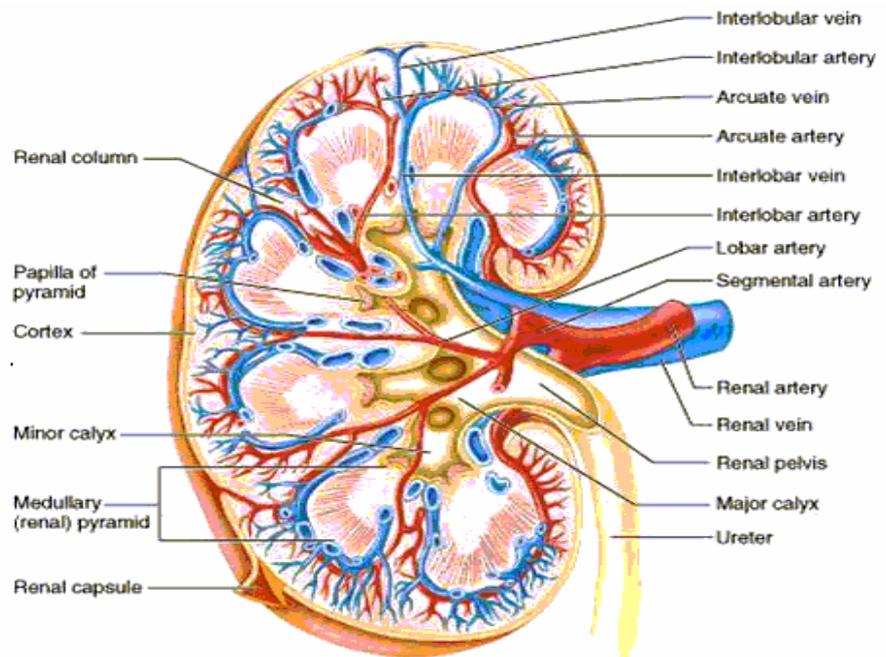junction, the interlobular arteries turn to follow a more horizontal course and form arterial arches (the arcuate arteries) across the bases of the pyramids. From the arcuate arteries, the interlobular arteries arise and run outward through the cortex. The interlobular arteries are branched into the afferent arterioles which carry the blood to the glomeruli.

In Bowman's capsule, each afferent arteriole breaks into glomerular capillaries which coalesce again forming efferent arteriole. Afferent arteriole is short with a diameter twice than that of the efferent arteriole to maintain a relatively high pressure of the blood entering the glomerulus. In the cortical region, the efferent arteriole breaks into a network of capillaries surrounding the convoluted tubules (peritubular capillaries), and thence to interlobular veins. The latter drain into the
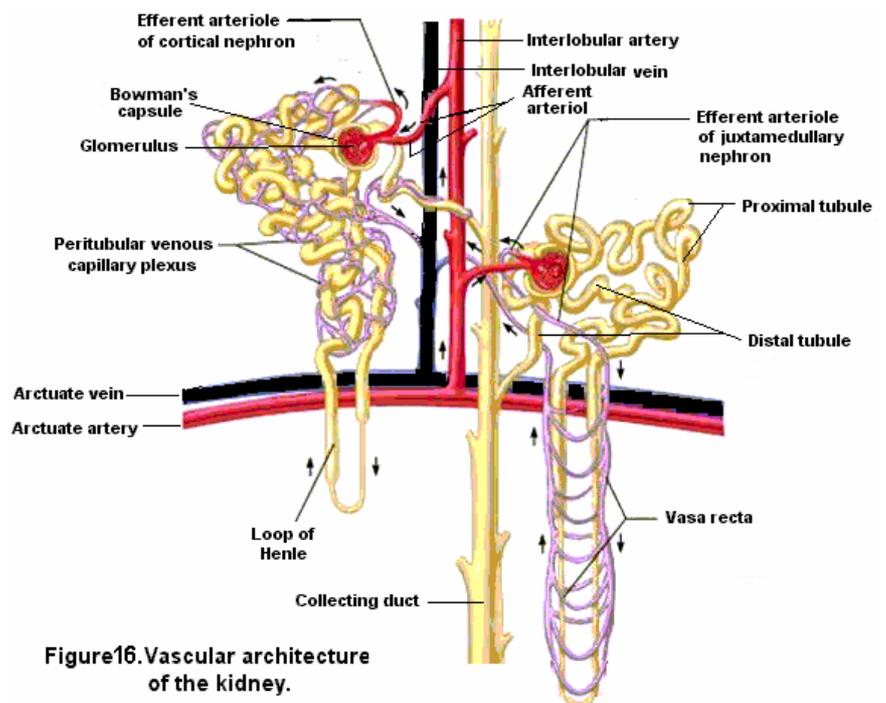
Figure15. Blood supply to the kidneys

Figure16.Vascular architecture of the kidney.

arcuate veins and thence into the renal vein.

The efferent arterioles derived from the juxtamedullary glomeruli do not fed into peritubular capillaries plexus, but each one divides into a group of straight vessels called the *vasa recta*, which descend toward the apex of the pyramids, following a course similar to the loop of Henle, and then return back to the corticomedullary junction and empty into arcuate veins (Fig. 16). The descending and ascending limbs of the *vasa recta* lie in a manner ideally suited for diffusional exchanges between inflowing and outflowing blood (counter-current fashion), which has a functional significance in the formation of urine.

URINE FORMATION

Nephrons are responsible for the formation of urine. Nephron has three functions: (I) Filtration of water and dissolved substances from the blood into Bowman's capsule and this occurs through the glomerulus. (II) Reabsorption of water and solutes through the uriniferous tubules. (III) Secretion into the lumen of the tubule of some substances formed by the tubule cells or which are circulating in peritubular venous capillaries surrounding the distal tubule.

(I) Glomerular filtration of water and solutes from the blood

About 1,300 ml blood flows through the kidney per minute (They contain about 700 ml plasma and 600 ml of cells). From this plasma volume (700 ml), about 125 ml of fluid is filtered by nephrons per minute and enter into the Bowman's capsule. This means that the two kidneys produce 180 L filtrate per 24 hr, of which 178.5 L are reabsorbed and the remaining 1.5 L was excreted as urine.

The primary urine in Bowman's capsule, glomerular filtrate, is identical in composition with the plasma in respect of electrical conductivity, alkalinity; crystalloid osmotic pressure; concentration of urea, glucose, inorganic phosphates, chlorides, etc. but it is devoid of proteins (and other colloids). Glomerular filtration is a passive process, i.e. it does not need energy and it is under the pressure force.

Mechanism of glomerular filtration

Glomerular filtration is due to physical factor but is not due to vital process as evidenced by the fact there is no increased oxygen consumption or heat production. The mechanism of glomerular filtration therefore depends on:
(1) Mean blood pressure in the glomeruli.
(2) Colloidal osmotic pressure.
(3) Pressure in the Bowman's capsule.
(4) Integrity of the basement membrane.

(1) Mean blood pressure in the glomeruli

Blood pressure (hydrostatic pressure) in glomeruli capillaries is measured to be 55 mmHg in normal condition. This pressure is higher in comparison to the pressure in the other capillaries. The cause may be due to (i) direct origin of renal artery from the aorta and its short length, (ii) The diameter of the efferent arteriole is half that of afferent arteriole causing stagnation of the blood, favouring raised blood pressure. This pressure is the filtration pressure (Fig. 17).

The blood flow through the kidneys are regulated in spite of the variations of the systemic blood pressure (autoregulation of renal blood flow).
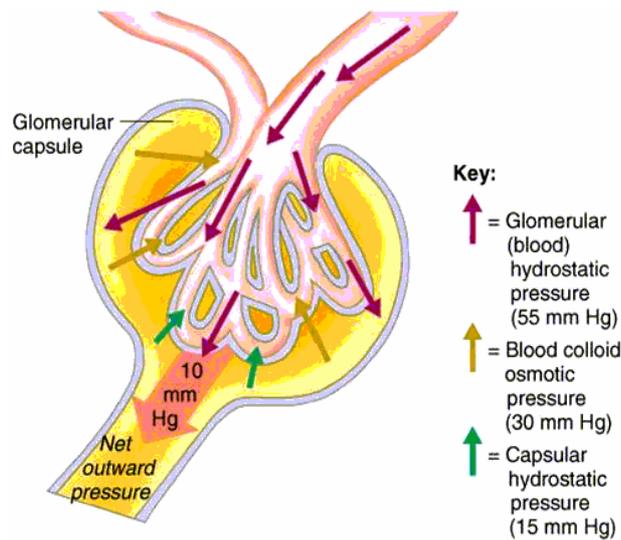

Figure 17. Mechanism of glomerular filtration.

(2) Colloidal osmotic pressure
    Colloidal osmotic pressure is exerted mainly by the plasma proteins and it is equivalent of a hydrostatic pressure varying from 25-30 mm Hg. It opposes the filtration pressure since it resists the filtration of water from plasma to the Bowman's capsule fluid. Experimentally, it can be shown that sudden dilution of the plasma proteins by the administration of isotonic sodium chloride solution produces an increase in the glomerular filtration and urine flow, because dilution of the plasma proteins decrease the resistance force created by colloidal osmotic pressure against filtration pressure.
(3) Pressure in the Bowman's capsule
    Hydrostatic pressure of the fluid in Bowman's capsule is about 15 mm Hg. It increases in case of obstruction in the tubules and ureter.
 (4) Permeability of the basement membrane
    In normal state, substances of smaller molecular weight like hemoglobin (68,000) can pass through the basement membrane. The permeability of the basement membrane increases in many abnormal conditions such as anoxia, inadequate blood supply, action of toxic agents, and in many diseases processes. Increased permeability of the basement membrane allows serum albumin (mol. wt. 70,000) and serum globulin (mol. wt. 1,70,000) to pass through the membrane.
Effective filtration pressure
    Blood pressure in glomeruli capillaries which is measured to be 55 mmHg is called filtration pressure. The forces opposing the filtration pressure are:
(i) Colloid osmotic pressure of plasma proteins (30 mm Hg).
(ii) Hydrostatic pressure exerted by the fluid in the Bowman's capsule (5 mm Hg).

So**, Effective filtration pressure** = Glomerluar pressure – (Colloidal pressure + Capsular pressure) = 55 – (30 +15) = 10 mm Hg.

It is noticed that the amount of filtrate that is formed is directly proportional to the net filtration pressure. The net filtration pressure is directly proportional to glomerular BP and inversely to capsular hydrostatic pressure and capillary osmotic pressure. The balance of these forces will determine how much filtrate is made per unit time.

Glomerular filtration rate (GFR)

The glomerular filtration rate (GFR) is the total amount of filtrate formed per minute by the kidneys. This is directly proportional to the effective filtration pressure. It is important to maintain a constant GFR, as the rate at which the filtrate is flowing through the renal tubules directly influences the reabsorption of water and other substances from the filtrate.

Glomerular capillaries have higher hydrostatic pressure and a permeability 100 times that of ordinary limb capillaries. Pore sizes of the glomerular capillary wall allow the particles with mol. wt. 68,000 or less to pass. The effective area available for filtration depends upon the number of glomeruli active at any time and the number of functioning capillaries in each glomeruli. It appeared that GFR is determined on a basis of the pressure force, not the blood flow or plasma flow, thus the GFR has no any particular relationship to the cardiac output. GFR is steadily maintained as long as blood pressure is between 80 mmHg and 180 mmHg.

glomerular filtration regulation

In humans, GFR is maintained at a relatively constant rate by renal myogenic autoregulation and (the intrinsic system), the rennin-angiotensin system (tubuloglomerular feedback), and neural control. Here, we discuss the first two factors
.

Renal myogenic autoregulation

The kidney regulates GFR independently of any outside tissue, organ, or chemical - hence the term autoregulation. As a result of myogenic autoregulation, an increase in systemic blood pressure does not cause an increase in either net filtration pressure or GFR. This is because, an increase in systemic blood pressure will cause the afferent arteriole to constrict. This will prevent an increase in net filtration pressure from occurring - since less of this "high pressure blood" will be arriving at the glomerulus. On the other hand, a decrease in systemic blood pressure will result in dilation of the afferent arteriole. This lets more "low pressure blood" arrive at the glomerulus, and net filtration pressure and GFR are thus maintained. Since this process is primarily mediated by arteriolar smooth muscle, it is termed myogenic.

Tubuloglomerular feedback

Now, let's consider tubuloglomerular feedback. If blood pressure or blood volume drops, GFR can drop as well. Low GFR means that filtrate will flow through the tubules relatively slowly. Thus by the time the filtrate has reached the distal convoluted tubule, a lot of the Na+ in the filtrate will have been reabsorbed. So, low [Na+] of the filtrate in the distal convoluted tubule is considered as indicative of low GFR.

There is a portion of the distal convoluted tubule that abuts the afferent arteriole. At that region of the distal tubule, there are tall, closely-packed tubule cells that collectively are known as the macula densa (Fig. 5). These macula densa cells are chemoreceptors that are responsible for measuring filtrate [Na+] and hence measuring GFR, blood pressure, and blood volume. In the afferent arteriole at this point are specialized smooth muscle cells known as juxtaglomerular cells. These cells contain large amounts of secretory granules containing renin.

If the macula densa cells are exposed to filtrate with low [Na+] they'll respond. They will release a chemical that'll cause vasodilation of the afferent arteriole and vasoconstriction of the efferent arteriole. This will increase blood pressure in the glomerulus and thus increase net filtration pressure and GFR.

Concentration of sodium in filtrate will also cause the juxtaglomerular cells to secrete renin. As mentioned before, renin is an enzyme that cleaves the plasma protein angiotensinogen into angiotensin I. Angiotensin I is converted into angiotensin II by Angiotensin converting enzyme, an enzyme associated with capillary endothelium. Angiotensin II is a potent vasoconstrictor and thus increases blood pressure. The increase in blood pressure will increase net filtration pressure and GFR.

Angiotensin II also prompts the adrenal cortex to release aldosterone and the posterior pituitary gland to release antidiuretic hormone (ADH). Aldosterone will cause certain distal convoluted tubule cells to reabsorb more Na+ and thus more water. ADH will cause the collecting duct to increase its reabsorption of water. This water will be reabsorbed into the peritubular capillaries. Thus both blood volume and blood pressure can be increased. By increasing blood pressure and blood volume, GFR is kept in that homeostatic state.

It should also be noted that the juxtaglomerular cells themselves act as mechanoreceptors (baroreceptors) that measure blood pressure within the afferent arteriole. If afferent arteriole blood pressure drops below 80 mmHg they begin to release renin (without any input from the macula densa cells).

Measurement of GFR
 The difference between the rate at which a substance is filtered at the glomeruli (mg/min) and the rate at which it is excreted in the urine (mg/min) represents the rate at which the substance is removed from or added to the urine as the latter traverses the renal tubules. The rate at which a substance is filtered at

the glomeruli is given by the product of its concentration in the plasma (P) (i.e., the unbound substance in the plasma water) and the rate of formation of glomerular filtrate (GFR). The rate at which it is excreted in the urine is easily determined as the product of urine flow (V) and the concentration of the substance in the urine (U). Thus, the measurement of the GFR plays a central role not only in the evaluation of the glomerular filtration itself, but in assessment of the processes of tubular reabsorption and secretion.

To calculate the GFR, consider a substance X which would be infused in the blood having the requisite characteristics of 1) being freely filterable at the glomeruli. 2) being neither reabsorbed from nor excreted into the tubule lumen.

Urine flow (V) = volume/time of collection = ml/min.

If the concentration of a substance X in the urine is U_x (mg/ml), the rate of excretion is given by the product of $U_x V$ (mg/min).

Because this substance is neither reabsorbed nor secreted, the concentration of the substance in the filtrate is the same as the concentration in the plasma (P_x).

Thus, the rate of filtration of X is equal to the product of GFR x P_x (mg/min).

As the rate of excretion is equal to the rate of filtration,

So, GFR x $P_x = U_x V$

GFR = $U_x V / P_x$

On basis of the above information regarding the substance which has the requisite characteristics for measuring of the GFR, **inulin**, a starchlike polymer (mol. wt. 5,200) obtained from dahlia tubers, is generally accepted. This compound is not metabolized in the body, and it can appear in the urine only if filtration takes place. It is never excreted by cellular transport or secretion, for it is inert to all known active transport processes (i.e. it is not reabsorbed, stored or secreted by the kidneys).

Using inulin, GFR in an intact animal cab be determined as follows: (1) A suitable amount of inulin is injected in the animal. Inulin is infused continuously throughout an experiment in order to maintenance of the plasma concentration of inulin constant, instead of declining continuously during the observation period. (2) Urine is collected during a known period of time and its volume is measured. (3) Inulin concentration in both urine and plasma is determined.

Renal clearance

Associated with GFR is the concept of renal clearance. This refers to the volume of plasma that is cleared of a specific substance in a defined amount of time (usually fixed at a minute). Renal clearance tests are performed to determine the GFR, and also to detect glomerular damage and to follow the progress of renal disease. Renal clearance rate (RC) in ml/min is calculated by the following equation:

RC = UV/P

Where U = the concentration (mg/ml) of the substance in the urine, V = the flow rate of urine formation (ml/min), and P = the concentration of the same substance in the plasma.

In practice, to determine the clearance, inulin is administered intravenously, followed by a sustaining infusion to keep the arterial plasma level constant. After the inulin has equilibrated with body fluids, an accurately timed urine specimen is collected and a plasma sample obtained halfway through the collection. Plasma and urinary inulin concentration are determined and the clearance is calculated.

The clearance of inulin in a man of average size under normal basal conditions averages slightly in excess of 125 ml/minute. In order to compare individuals of varying size, the clearance values are normalized by correcting them to what is generally considered as average normal value of body surface area, 1.73 m^2. Women have glomerular filtration rats about 10% lower than those found in the men even after correction to the same value of the surface area. Knowing the renal clearance value of a drug is essential when determining the dose size and frequency. For example, a drug with a high renal clearance value, the drug must be given frequently in fairly large doses.

RENAL BLOOD FLOW

In a resting adult, the kidneys receive 1.2-1.3 L of blood per minute (about 25% of the cardiac output). Renal blood flow can be measured with electromagnetic or other types of flow meters. Since the kidney filter plasma, the term renal plasma flow is used and can be determined by measuring the amount of a given substance excreted per unit of time divided by the renal arteriovenous difference as long as the amount in the red cells is unaltered during passage through the kidney.

This means that: $F = R/(A - V)$

Where F is the flow, R the rate of removal, an A and V the concentration in arterial and venous blood respectively.

A substance which can be used in the determination of renal plasma flow has such characters: (i) Its concentration in arterial and renal venous can be measured; (ii) It is not metabolized or stored, i.e. all of the PAH removed from the blood is excreted in the urine; and (iii) It is produced by the kidney from plasma and does not itself affect blood flow. For instance, *para*-aminohippuric acid (PAH) has been found to be a best substance which is used for calculating the renal plasma flow. As PAH is filtered by the glomeruli and secreted by the tubular cells, its extraction ratio (arterial concentration minus renal venous concentration divided by arterial concentration) is high. So, when PAH is infused at low doses, 90% of the PAH in arterial blood is removed in a single circulation through the kidney, and this leads us to calculate the renal plasma flow *by dividing the amount of PAH in the urine by the plasma PAH level, ignoring the level in the renal venous blood*. Peripheral venous plasma can be used because its PAH concentration is essentially identical to that in the arterial plasma reaching the kidney. The value obtained should be called *effective renal plasma flow* (ERPF) to indicate that level in renal venous plasma was not measured. In human, ERPF averages about 625 ml/min. In applying the previous equation to the measurement of renal plasma flow using PAH, the yield:

$RPF = U_{PAH}V/(P^A_{PAH} - P^{RV}_{PAH})$

$ERPF = U_{PAH}V/P_{PAH} = $ Clearance of PAH (C_{PAH})

(*Note that: ERPF for PAH is equal to C_{PAH} when PAH is given at low doses, so the plasma level is maintained at low concentration. But, if the plasma level is increased, the extraction becomes less complete and the clearance falls further below the true plasma flow*).

From the renal plasma flow, the renal blood flow can be calculated by dividing by 1 minus the hematocrite:

Hematocrite (Hct) = 45%

Renal blood flow = RPF x 1/1 – Hct

(II) Reabsorption of water and solutes through the uriniferous tubules

Of the ~125 ml of plasma filtered by the glomeruli, 124 ml is reabsorbed during passage through the renal tubules. Tubular reabsorption is a transepithelial process carried out in the proximal tubule, loop of Henle, distal tubule, and the
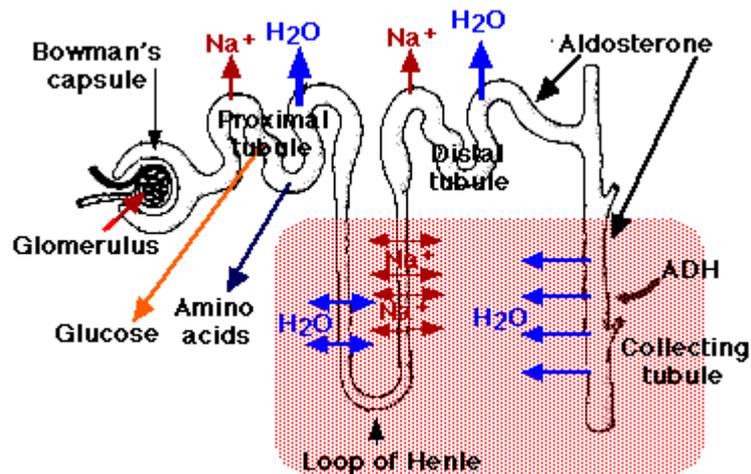

Figure 18. The method of urine formation.

collecting ducts. The process of tubular reabsorption of different substances (water, ions, and nutrients) may result from either active cellular transport (via cotransporters) or passive back diffusion (via simple diffusion, facilitated diffusion, and osmosis). For example, reabsorbed urea and about 85% of water leak through the lumen as a result of passive diffusion. But the reabsorption of most solutes, e.g. sodium and glucose is an active process (Fig. 18).

The efferent arterioles give rise to the peritubular capillaries. The peritubular capillaries run alongside and around the renal tubules and drain into the venules of the renal venous system (Figures 15 and 16). They are low pressure, porous capillaries that readily take up solutes and water from renal tubule cells during the process of reabsorption. Note that in juxtamedullary nephrons, the efferent arterioles give rise to the vasa recta - bundles of long straight vessels that run beside the loops of Henle deep into the medulla (Fig. 16).

Proximal tubular reabsorption
Glucose and amino acids

These substances normally are completely reabsorbed by active transport in the proximal tubule (Fig. 19).. Glucose is a threshold substance; i.e. the tubular cells have a limited capacity to reabsorb it, so a rise of plasma glucose level above 180 mg/100ml causes glycosurea (presence of glucose in urine). Plasma level of glucose at which glycosurea starts is known as renal threshold.

Sodium and chloride

   Na$^+$ ions are the most abundant ion in the filtrate. Since they are more concentrated in the filtrate than in the inside of the renal tubule cells, they passively flow down their gradient into the cells. Na$^+$ ions are then actively pumped out through the basolateral membrane by Na$^+$/K$^+$ pumps (cotransport " symport" with other solutes or by a Na$^+$-H$^+$ antiporter) (Fig. 19). This ion then diffuses into the peritubular capillary.

   About 65% of filtered sodium is reabsorbed from the filtrate in normal state by. The percentage concentration of sodium throughout the proximal tubule

Figure 19. Processes of reabsorption in the proximal convoluted tubule.
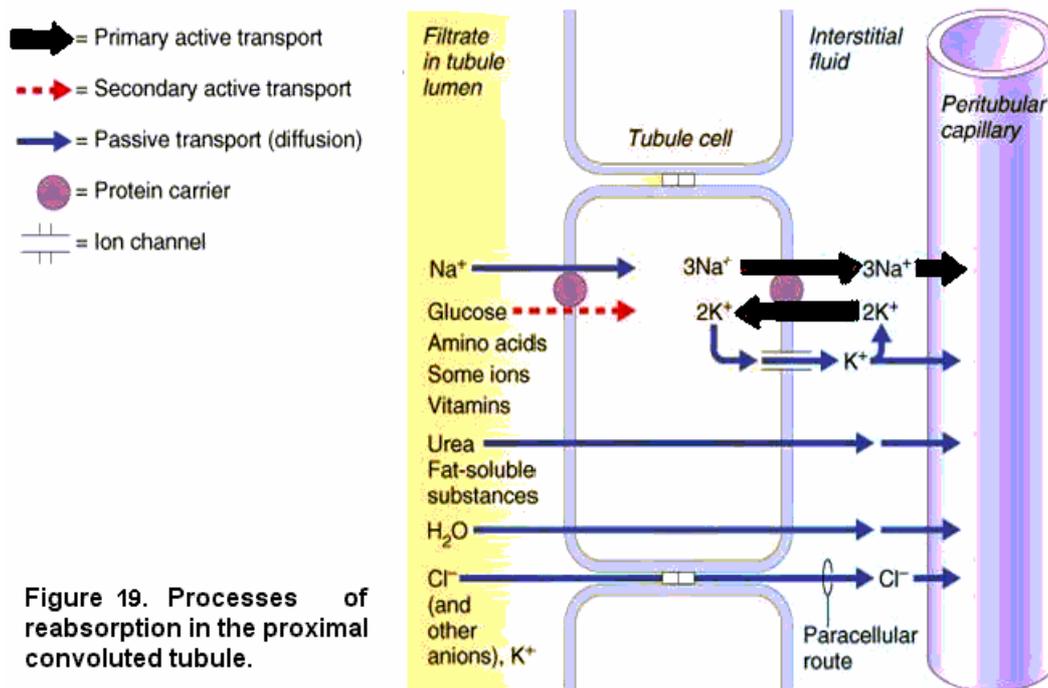
segment does not change, because water reabsorption follows sodium passively. Chloride as an anion is reabsorbed in accompanied with the cation sodium (about 50% of the Cl$^-$ is reabsorbed in proximal tubule).

Water

   Normally, 65% of the filtered water in the proximal tubule is reabsorbed (obligatory). The process of reabsorption is completely passive, because water movement is coupled with sodium transport (Fig. 19).

Bicarbonate

   Normal bicarbonate reabsorption in the proximal tubule participates in the adjustment of the pH of the tubular fluid to be the same as that of the glomerular filtrate. About 90% of the HCO$_3^-$ is reabsorbed in the proximal tubule. The cyclic mechanism of bicarbonate reabsorption can be summarized in Fig. (20) as follows:

1. H$^+$ is secreted by the tubule cell into the lumen and combines with filtered HCO3$^-$ to form carbonic acid (H$_2$CO$_3$). Secretion of H$^+$ is accompanied with Na$^+$ reabsorption by the tubular cells.

2. Urinary H_2CO_3 dissociates to CO_2 and water, the reaction in the proximal tubule is catalyzed by carbonic anhydrase on the luminal surface of the tubular cell.

3. CO_2 diffuses back into the tubular cell, because its partial pressure in the urine rises.

4. In the tubular cell, much of this CO_2 combines with water to form H_2CO_3 by the catalysis of the enzyme carbonic anhydrase.

5. H_2CO_3 then is ionized to $HCO3^-$ and H^+. The latter is secreted out again, and former   ($HCO3^-$) enters the blood together with Na^+.

Note that: *The $HCO3^-$ absorbed into the blood has not actually been absorbed from the tubular fluid because the tubular cells are impermeable to it. $HCO3^-$ filtered is reabsorbed from the tubular fluid as CO_2, then hydrated and ionized forming $HCO3^-$ inside the tubular cell.*
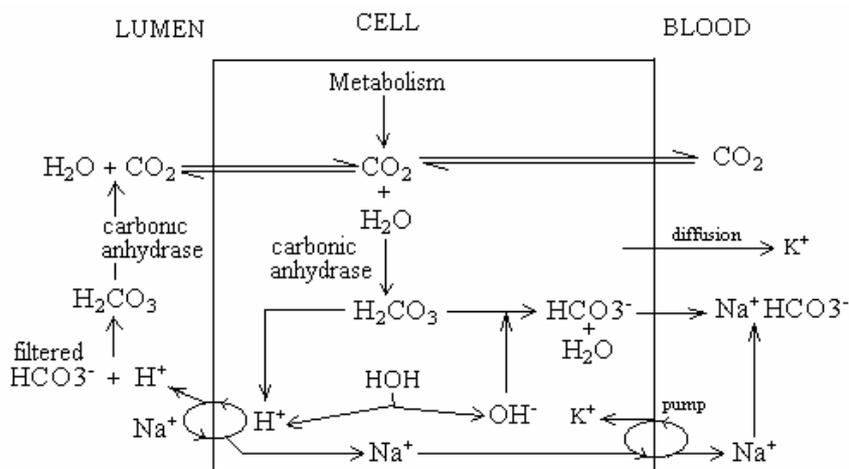

Figure 20. Hypothetical cellular processes in the secretion of hydrogen ion in exchange for sodium. Note the production of bicarbonate from carbone dioxide produced by cell metabolism or reabsorbed from the tubular filtrate.

Phosphate

It is reabsorbed by an active process in the proximal tubule. $HPO_4^-/H_2PO_4^{--}$ must be reabsorbed in ratio 4/1 (the same ratio in the plasma) to maintenance of the pH of the proximal tubular fluid to be the same as that of plasma. Circulating parathyroid hormone controls phosphate excretion, i.e. excess hormone diminishes tubular reabsorption. Vitamin D also has some inhibitory effect on phosphate reabsorption.

Potassium

About 55% of filtered potassium is reabsorbed in the proximal convoluted tubule.

Urate

Normally, urate is probably completely reabsorbed in the proximal tubule. Normal urinary urate is derived from active tubular secretion.

Urea

A part from urea diffuses passively into the blood from the proximal tubule as water reabsorption increases its concentration in the filtrate. This diffusion is limited. Urinary urea can reach concentrations well above those in the blood.

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The loops of Henle

In the loop of Henle, water reabsorption is no longer coupled to solute reabsorption. Water can leave the descending limb, but not the ascending limb, and these permeability differences play an essential role in the kidney's ability to produce dilute and concentrated urine. The opposition of descending and ascending loops of Henle and their neighboring blood capillaries (vas recta) plays an important role in the production of concentrated or diluted urine by a phenomenon known as counter-current multiplication and counter-current exchange.

Counter-current multiplication and counter-current exchange

Generally, a counter-current system is a system in which the inflow runs parallel to, counter to, and in close proximity to the outflow for some distance. For example, the opposition of both the loops of Henle and *vasa recta* in the renal medulla. The function of this system involves two mechanisms:

(i) Counter-current multiplication which depends on the active transport of sodium and chloride out of the thick ascending limb of loop of Henle, the high permeability of its thin descending limb to water, and the inflow of tubular fluid from the proximal tubule, with outflow into the distal tubule. This system functions in the production of a gradient of increasing osmolality along the interstitia of medulla, and reduction in the urinary osmosis (Fig. 21).

(ii) Counter-current exchange which is a passive process and acts to maintain the counter-current multiplication. In this process, the ascending *vasa recta* reabsorbs water which diffuses from the descending limb of the vascular loop, whereas NaCl and urea diffuse out of the ascending limb of the vessel and into the descending limb along the osmotic gradient created by multiplication (Figure 16). By this means the urine is concentrated and the plasma diluted.

Function of loop of Henle (Counter-current multiplication)

The fluid which leaves the cortical proximal tubule and received by the descending limb of the loops of Henle is isotonic (300 milli osmoles) with the interstitia (peritubular) and has a similar electrolyte content to that of the glomerular filtrate, but it reduced to 25% of its original volume.

To maintain such osmotic balance, water and ions from the interstitial fluid (peritubular) are rapidly removed away by blood flowing through the cortical capillaries (counter-current exchange).

This isosmotic fluid passes down through the thick descending limb of the loop of Henle where a progressive increase in the osmotic gradient from 400 to 1200 milli osmoles is maintained in the interstitium from the cortex to the loop in the medulla by the sodium pumps operating in the ascending limb of the loop of Henle (Counter-current multiplication, Figure 21).

Hypertonicity due to increased sodium and chloride in the interstitium of medulla facilitates (i) water diffusion into the medullary interstitium from the tubular fluid, which passes down through the thin descending limb of the loop of Henle and (ii) passive diffusion of sodium and chloride into the descending tubular fluid from the hypertonic interstitium.

As the ascending limbs of the loops are impermeable to water, but permeable to sodium as well as chloride ions, the osmotic concentration in the ascending fluid progressively falls by moving the fluid toward the cortex until it becomes hypotonic to the surrounding cortical interstitial fluid when it enters the distal convoluted tubule. At this point, the volume of the tubular fluid is only some 15% (about 16 ml/min) of that of the original glomerular filtrate.

As the fluid passes in the loop of Henle, a further 25% of the Na^+, 10% of the water, 35% of the Cl^-, and 30% of the K^+ are reabsorbed. (However, it should be noted that K^+ is recycled - it is reabsorbed in the ascending limb and secreted from the descending limb). Na^+ is reabsorbed into the lumen via a Na^+-K^+-$2Cl^-$ symporter, a Na^+-H^+ antiporter, and via a paracellular route.
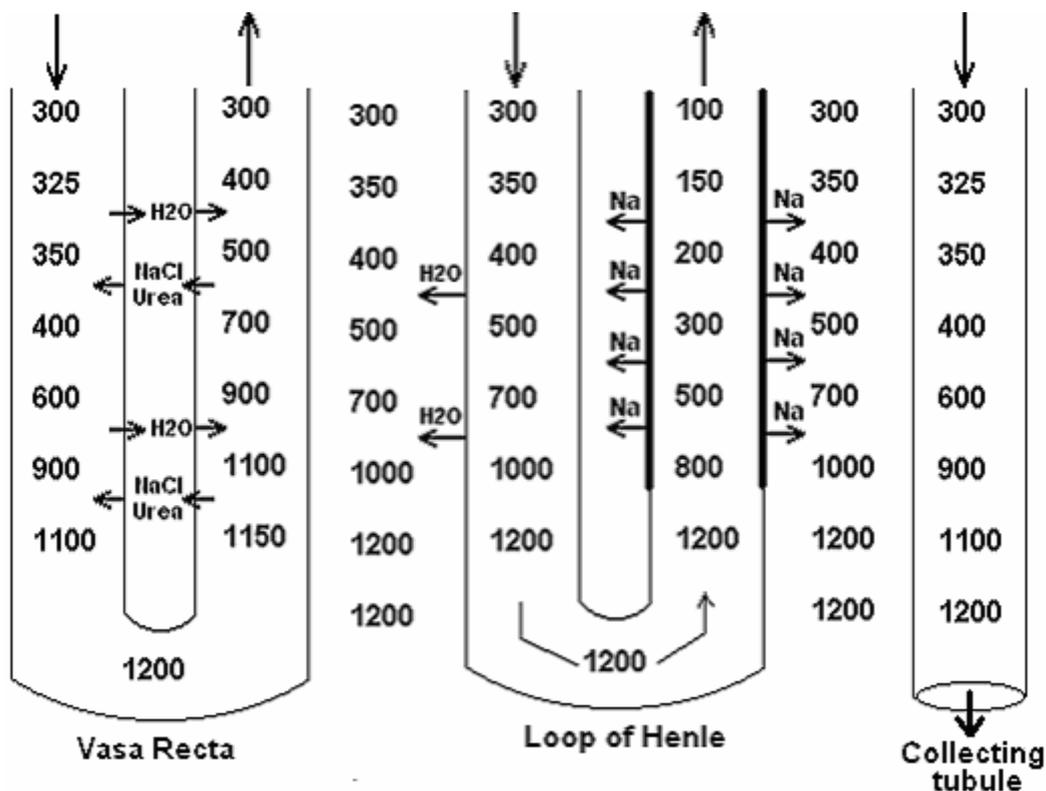

Figure 21. Mechanisms of urine concentration. Counter-current multiplication and counter-current exchange.

The distal convoluted tubule and collecting tubule

The distal convoluted tubules receive a hypotonic fluid from the ascending loops of Henle in a rate of about 16 ml/min, compare with the glomerular filtration rate which equals 127 ml/min. As the usual rate of urine formation is 1 ml/min, absorption of about 15 ml/min of the fluid occurs in both distal convoluted tubules and collecting tubules under the influence of antidiuretic hormone (ADH), which is secreted by the posterior lobe of the pituitary gland. Normally, the epithelial walls of the distal convoluted tubules and collecting tubules are impermeable to water in the absence of ADH.

As the fluid passes in the distal convoluted tubule it becomes isotonic due to water reabsorption secondary to active reabsorption of sodium in this segment

and in the ascending limb of loop of Henle and dependent upon circulating ADH. Water reabsorption in the distal convoluted tubule is facultative, and active reabsorption of sodium is stimulated by an adrenocortical hormone known as aldosterone. Chloride is absorbed passively with sodium.

The collecting tubules in the cortical region receive an isotonic fluid with a volume which is reduced to 5% of that of the original glomerular filtrate. In the collecting ducts urine is further concentrated and becomes hypertonic. This occurs by withdrawing the water from the collecting duct, under the influence of ADH, into the more hypertonic interstitium when the ducts traverse through the medullary layers of successively higher osmotic pressure. At the tips of the papillae, fluid in the ducts becomes more concentrated, and its osmotic concentration approaches that of the interstitial fluid, 1200 milli Osmolar (Figure 21). The fluid then enters the renal pelvis and is excreted as urine.

The sodium and chloride removed from the ascending limbs of the loops of Henle, and the water removed from the descending limbs and the collecting ducts are removed by blood circulation in the capillaries in the medulla and papillae (counter-current exchanger). This mechanism actually acts to maintenance of the osmotic gradient through the medullary interstitium.

Regulation of urine composition by ADH and aldosterone
ADH: It is called anti-diuretic hormone and increases the permeability of the distal tubule and collecting duct to water. If ADH is not present the membranes are impermeable to water. This results in less water passing back to the blood and a greater volume of urine produced. When ADH is present water is reabsorbed into the blood of the peritubular network by active transport and the urine produced is more concentrated. ADH secretion is controlled by the Hypothalamus. There are receptors here which monitor the salt concentration in the blood. When the solute (e.g. salt) concentration is high, the hypothalamus sends messages to the posterior pituitary which secretes ADH. When the solute concentration is low the hypothalamus stops sending messages to the posterior pituitary and the secretion is stopped.

Mechanism of action of ADH: Transmembrane channels made of proteins called aquaporins are inserted in the plasma membrane greatly increasing its permeability to water. (When open, an aquaporin channel allows 3 billion molecules of water to pass through each second.). Insertion of aquaporin-2 channels requires signaling by the ADH (also known as arginine vasopressin or AVP). ADH binds to receptors (called V2 receptors) on the basolateral surface of the cells of the collecting tubules. Binding of the hormone triggers a rising level of cyclic adenosine monophosphate (cAMP) within the cell. This "second messenger" initiates a chain of events culminating in the insertion of aquaporin-2 channels in the apical surface of the cell.

Aldosterone: It controls the level of Na^+ and K^+ in the blood by causing reabsorption of sodium (Na^+) and excretion of potassium (K^+) by the kidney. The hormone causes the principal cells of the collecting ducts to open or produce

more Na^+ channels and more Na^+-K^+ transporters. In contrast to aldosterone, atrial natriuretic peptide, a hormone released by atrial myocytes under conditions of raised blood pressure, inhibits Na^+ reabsorption in the collecting ducts.

What causes Aldosterone to be secreted

If the blood pressure drops (see above) it is sensed by receptors in the Juxtaglomerular apparatus. The Juxtaglomerular apparatus then secretes a hormone called renin. Renin converts angiotensinogen (a plasma protein produced by the liver) to angiotensin. Angiotensin does two things: it constricts the blood vessels which raises the blood pressure and it causes the release of aldosterone by the adrenal cortex. The aldosterone then travels to the kidney where it acts on the distal tubule and collecting duct to increase Na^+ reabsorption.

Diabetes insipidus

This disorder is resulted from (1) Insufficient secretion of ADH, (2) Inheritance of two mutant genes for the ADH receptor (V2), and (3) Inheritance of two mutant genes for aquaporin-2. Diabetes insipidus is characterized by: excretion of large amounts of a watery urine (as much as 30 liters — each day, exceed 16 ml/min) and remitting thirst.

Liddle's Syndrome

The most obvious effect of this rare, inherited disorder is extremely high blood pressure (hypertension). It is caused by a single mutant allele (therefore the syndrome is inherited as a dominant trait) encoding the aldosterone-activated sodium channel in the collecting tubules. The defective channel is always "on" so too much Na^+ is reabsorbed and too little is excreted. The resulting elevated osmotic pressure of the blood produces hypertension.

Reabsorption of urea

Urea moves passively out of the proximal tubule, but except for the inner medullary portion of the collecting duct, the rest of the tubular epithelium is virtually impermeable to this compound. Consequently, urea is increasingly concentrated in the fluid as water is removed in the loop and distal tubule. In the inner portion of collecting duct, urea moves into the interstitium of the pyramids, adding to the hyperosmolarity.

It has been shown that the permeability of the distal and collecting tubule toward water and solute controls the passive reabsorption of urea from the tubular fluid. When hypotonic urine is formed in the absence of ADH, urea moves from the interstitium into the tubular lumen, whereas with the normal hypertonic urine in the presence of ADH, urea is concentrated and absorbed into the hypertonic interstitial fluid, and its excretion is at its lowest.

The counter-current of fluid within the loop of Henle and the *vasa recta* traps urea within the medullary interstitial fluid (just as it traps NaCl), so the concentration of urea rises towards the tip of the papillae (this mechanism is known as counter-current exchanger, figure 21) leading to rising the osmotic concentration of the medullary interstitial fluid, hereby more fluid is reabsorbed

from the collecting duct and concentrated urine is produced. This explain the fact that the capacity to concentrate urine is highest on a high protein diet where a large amount of urea is formed and filtered.

(III) Tubular secretion

The substances secreted into the tubular lumen either formed by the tubule cells or which are circulating in peritubular venous capillaries surrounding the distal tubule. They are include:

Secretion of H^+

Acidification of the urine occurs by secretion of H^+ from the tubular cells into the lumen in proximal tubules, distal tubules, and collecting ducts.

In the proximal tubule

The reaction for H^+ secretion in proximal tubules involves Na^+- H^+ exchange (Figure 20). In this mechanism, Na^+ is removed from the cells into the interstitium by the action of Na^+- K^+ ATPase leading to lower its intracellular concentration. This causes Na^+ to enter the cell from the tubular lumen, with coupled extrusion of H^+. The H^+ comes from intracellular dissociation of H_2CO_3, and the $HCO3^-$ that formed diffuses into the interstitial fluid. It appears that, for each H^+ ion secreted, one Na^+ ion and one $HCO3^-$ ion enter the interstitial fluid. The enzyme carbonic anhydrase catalyzes the formation of H_2CO_3, so the drugs that inhibit this enzyme decrease both secretion of acid by proximal tubules and the reactions which depend on it.

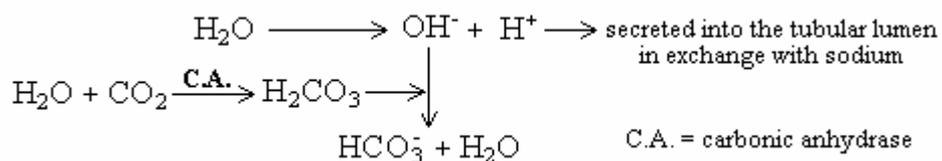### In the distal tubules and collecting ducts

In contrast to the mechanism mentioned above in the proximal tubules, H^+ secretion in both distal tubules and collecting ducts is relatively independent of Na^+ in the tubular lumen. So, another mechanism involves an ATP-driven proton pump is used for H^+ secretion in this part of the renal tubules. Aldosterone acts on this pump to increase distal H^+ secretion.

The I cells in both distal tubules and collecting ducts secrete acid. They contain abundant carbonic anhydrase and numerous tubulovesicle structures. The pump of H^+ secretion (ATPase) is located on the tubulovesicle as well on the luminal cell membrane. In case of acidosis, the number of H^+ pumps is increased by insertion of these tubulovesicles into the luminal cell membrane. I cells contain in their basolateral membranes a protein which may function as a Cl^-- HCO_3^- exchanger for the transport of HCO_3^- to the interstitial fluid.

Factors affecting acid secretion

(i) Intracellular P_{CO2}

When the P_{CO2} is high, as in case of respiratory acidosis, more intracellular H_2CO_3 is formed and becomes available to buffer the hydroxyl ions. This results in enhancement of H^+ secretion (See the reactions below). In case of reduction of intracellular P_{CO2}, the reverse is true.

$$H_2O \longrightarrow OH^- + H^+ \longrightarrow \text{secreted into the tubular lumen in exchange with sodium}$$

$$H_2O + CO_2 \xrightarrow{C.A.} H_2CO_3 \longrightarrow HCO_3^- + H_2O \qquad C.A. = \text{carbonic anhydrase}$$

(iii) Activity of carbonic anhydrase

Inhibition of carbonic anhydrase activity causes reduction in the acid secretion, because the production of H_2CO_3 by the action of mentioned enzyme is decreased (See the reactions above).

(iv) Aldosterone and other adrenocortical steroids

Aldosterone and other adrenocortical steroids enhance tubular reabsorption of sodium and increase the secretion of acid and potassium.

Secretion of ammonia

Principally, ammonia (NH_3) is produced in the cells by the conversion of glutamine to glutamate. The reaction is catalyzed by the enzyme glutaminase, which is abundant in the renal tubule cells, especially the distal tubule and collecting duct cells. NH_3 is further formed from glutamate when it converted to α-ketoglutarate in the presence of the enzyme glutamic dehydrogenase (Figure 22). Oxidative deamination for some L-amino acids (e.g. glycine, alanine, leucine, aspartic acid, ..etc) present in the cell by the catalysis of enzyme deaminase leads also to produce NH_3. Glutamine and aspargine appear to be the major source of ammonia present in the urine.

Cells are permeable to NH_3 (because it dissolves in the lipid) but not to the ionized ammonium (NH_4^+). NH_3 thus diffuses into the tubular lumen and reacts with H^+ to form NH_4^+, the latter remains in the tubule lumen and excreted in the urine. The process by which NH_3 is secreted into the tubular fluid is called nonionic diffusion. The diffusion rate depends upon the urine pH and facilitated by the change of NH_3 to NH_4^+ in the urine (Figure 22). So, in case of acidosis (acid urine, pH is low), urinary excretion of NH_4^+ increases, because more ammonia secretes into the tubular lumen and combines with H^+, whereas in case of alkalosis (pH is high) excretion of ammonium is ceased.
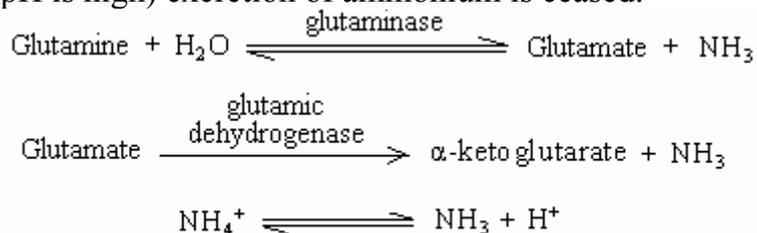
$$\text{Glutamine} + H_2O \xrightleftharpoons{\text{glutaminase}} \text{Glutamate} + NH_3$$

$$\text{Glutamate} \xrightarrow{\substack{\text{glutamic} \\ \text{dehydrogenase}}} \alpha\text{-ketoglutarate} + NH_3$$

$$NH_4^+ \rightleftharpoons NH_3 + H^+$$

Figure 22. Major reactions involved in ammonia formation in the kidney.

If the blood is acidic (Low pH)

If the blood is acidic the kidney brings it back to normal by excreting hydrogen ions (H^+) and ammonia, while reabsorbing sodium ions and bicarbonate ions. The hydrogen ions and ammonia are excreted during tubular excretion at the distal tubule.

If the blood is alkaline (high pH)

If the blood is alkaline, fewer hydrogen ions are excreted and fewer sodium and bicarbonate ions are reabsorbed.

Secretion of potassium

Much of filtered potassium ion is reabsorbed actively in the proximal tubules. However, potassium is added to the urine again in the distal convoluted tubule by

the secretory activity of the tubular cells in exchange for sodium, and this process is stimulated by aldosterone. The movement of K^+ is passive. The rate of K^+ secretion is proportionate to the flow rate of the tubular fluid through the distal tubule, i.e. with rapid flow there is no tendency to rise the K^+ concentration in the tubular fluid hence the secretion could be increased and vice versa. Normally, the amount of K^+ secreted is approximately equal to the K^+ intake, and potassium balance is maintained.

Potassium and hydrogen compete for this exchange in the distal tubule cells. So, deficiency of potassium (hypokalaemia) promotes the secretion and excretion of H^+ ion in the urine which may lead to alkalosis (plasma pH is elevated). Whereas excess of cellular potassium (hyperkalaemia), i.e. intracellular alkalosis, favours potassium secretion and excretion.

Creatinine

In man, creatinine is not reabsorbed but it is secreted by the renal tubule in small amounts. This compound is normally formed by the muscles and is of particular interest because it can be used in studies of renal function in a manner similar to inulin. It is formed in the body at a relatively constant rate, and monitoring its excretion allows study of renal function without injection of a foreign substance. Creatinine is apparently derived from the metabolism of the high-energy phosphate compound, phosphocreatine.

Certain exogenous substances

Certain substances such as PAH, diodone, mercurial diuretics, penicillin are secreted from the plasma into the proximal tubular fluid by active cellular work.

CONTROL OF ACID-BASE BALANCE AND URINARY ACIDIFICATION BY RENAL TUBULES

Fate of H^+ in the urine

Secretion of H^+ into the tubular fluid is limited and depends on the composition of tubular fluid. The transport mechanism functions until the pH of urine reaches 4.5 (the limiting pH). So, if there is no buffers that tied up H^+ in the urine, the limiting pH would be reached rapidly, and H^+ secretion would stop. The presence of buffer systems in the tubular fluid are therefore necessary for excretion of maximum amounts of H^+ (and correspondingly for maximum conservation of sodium ion).

Normally, there are three important buffering reactions in the tubular fluid remove the free H^+ and permit secretion of more acid (Figure 23). These are the reactions (i) with HCO_3^- to form CO_2 and H_2O, (ii) with dibasic phosphate (HPO_4^{2-}) to form monobasic phosphate ($H_2PO_4^-$), and (iii) with secreted NH_3 to form NH_4^+. Net quantity of free hydrogen ions in association with a buffer system in the urine is measured by the amount of alkali needed for returning acid urine to pH 7.4 (the pH of the plasma) and that is known as the *titrable acidity*. H^+ ions excreted by the kidney is measured by the sum of the titrable acidity and ammonium.

In the proximal tubule

   The concentration of HCO_3^- in both plasma and glomerular filtrate is normally about 27 mEq/L, whereas that of phosphate is only 1.5 mEq/L. This means that most of the H^+ ions secreted in the proximal tubule react with HCO_3^- to form H_2CO_3 (Figure 23). The latter (H_2CO_3) is broken down by the catalysis of the enzyme carbonic anhydrase into CO_2 and H_2O. Carbonic anhydrase presents in the brush border of the proximal tubule cells. CO_2 diffuses into the tubular cells where it reacts with H_2O forming H_2CO_3. By this mechanism for each one mole of HCO_3^- removed from the tubular fluid as CO_2, one mole of HCO_3^- diffuses from the tubular cells into the blood, even if it is not the same mole that disappeared from the tubular fluid. Actually, reabsorption of bicarbonate via this pathway (expenditure of H^+) would lead to: (i) Maintenance of pH of urine at 7.4, like that of the glomerular filtrate. (ii) Prevention of the loss of bicarbonate from the body. When the plasma HCO_3^- concentration is low, all the filtered HCO_3^- is reabsorbed, but when the plasma HCO_3^- concentration is high, i.e. above 26-28 mEq/L (the renal threshold for bicarbonate), HCO_3^- appears in the urine and the urine becomes alkaline.

   Due to the small concentration of phosphate in the proximal tubule, some of secreted H^+ ions react with the dibasic phosphate (Na_2HPO_4) to form monobasic phosphate (NaH_2PO_4). Secreted H^+ also reacts with NH_3 to form NH_4^+ in the proximal tubule. See Figure (23).
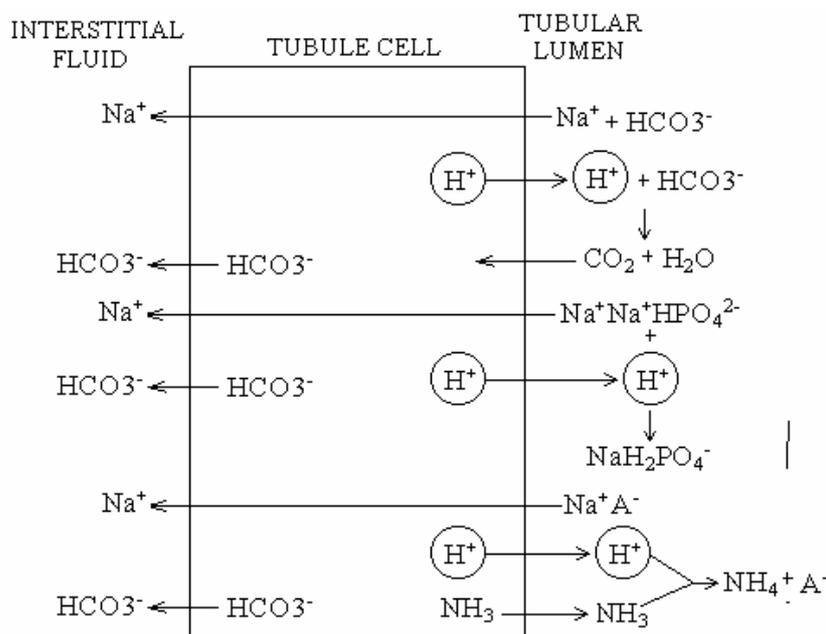

Figure 23. Fate of H2 secreted by the tubular cell into the lumen in exchange for Na+. **Top**: Reabsorption of filtered bicarbonate. **Middle**: Formation of monobasic phosphate. **Bottom**: Ammonium formation. Note that in each instance one Na+ and one HCO3- enter the blood circulation for each H^+ secreted. A-, anion.

In the distal tubule and collecting duct

   The phosphate, which escapes from the proximal tubule, is greatly concentrated in the distal tubule (15 mmol/L) and collecting duct due to the great

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reabsorption of water. Therefore, in these parts of renal tubules, most of secreted H^+ ions are removed by the reaction with the dibasic phosphate (Na_2HPO_4) to form monobasic phosphate (NaH_2PO_4). Note that, the pH of urine falls with the formation of NaH_2PO_4 (urine acidification). See Figure (23). Secreted H^+ also reacts with NH_3 to form NH_4^+ in the distal convoluted tubule.

Changes in pH along the nephrons

In the proximal tubule most of H^+ ions secreted consumed in the reabsorption of HCO_3^-. Remaining H^+ ions in the urine which present in association with the buffer system (particularly phosphate) produce only a small effect on the pH of the fluid in the proximal tubule. In distal tubule H^+ ion is secreted with the reabsorption of Na+, and its amounts are in excess in respect to that of HCO_3^- remained in distal fluid. Therefore, acidification of urine becomes true in the distal tubular lumen as well collecting duct, where the pH of the urine in these parts could differ largely from that in the cells.

PHYSICAL CHARACTERS OF URINE

Volume

The average urine volume is approximately 1.5 liter (1500 ml) per day. It varies from 600 to 2,500 ml daily. The variations in daily output of urine depend on: (a) water intake, (b) environmental temperature, (c) type of diet, and (d) mental and the physical state. More volume of urine was excreted in the winter than summer season, as perfuse sweating occurs in summer. Tea and caffee increase urinary volume. During sleep, about 50% of the urine occurs and the rest during walking state. When urine volume is 500-600 ml or less in 24 hours, it is called oliguria, but when it is less than 150 ml it is called anuria. Urine volume more than 2 liters per day is called polyuria.

Specific gravity

It ranges from 1,002 to 1,035, depending on the solute concentration.

Osmolarity

It represents the total solute contents present in the urine and expressed as Osmoles/L, so there a good relationship between it and the specific gravity. Osmolarity of the urine ranges from 50 to 1,400 mOsmol/L or 0.5 to 1.4 Osmoles/L, compared to 285 mOsmol/L in plasma. This represents the efficient functioning of the kidney. Osmolarity varies inversely with the urine volume. The minimum volume required for excretion of all the solutes including the nitrogenous waste is known as obligatory urine volume (680 ml in 24 hours).

Reaction

On a mixed diet, the reaction of urine is acidic. The pH varies from 4.7 to 8 with a mean pH 6. About 25 mEq H^+ ions (titrable acidity) and 250 ml of 0.1 N acid are excreted per 24 hours. High protein intake increases acidity, due to oxidation of S and P of amino acids. Vegetarian diet or diet mostly consisting of fruits and vegetables results in alkaline urine. Metabolic acidosis as in case of fever and starvation lead to acid urine. Urine reaction becomes alkaline if it is kept for sometime, because urea is converted to ammonia. Alkalosis and excessive vomiting lead to alkaline urine.

Color

The color of urine is straw yellow or pale yellow. When excessive urine is passed, the color becomes transparent. It is due to urochrome, a pigment, and also due to urobilin and haematoporphyrin and uroerythrin (derived from melanuria) content. Fever causes dark urine or brownish. In the presence of bile pigments, urine becomes yellowish green or deep yellow or brown. The color of urine contains blood or haemoglobin is smoky. Some drugs make the urine yellow as furadantin or furoxone. Methylene blue gives urine blue color, while riboflavine gives greenish-yellow fluorescence. Urine is transparent when acid, but becomes turbid when becomes alkaline due to the precipitation of alkaline phosphate. Very strong acidity makes urine pink, due to precipitation of uric acid salts.

Odour

The smell of fresh urine is aromatic. After a few minutes, the smell of urine becomes pungent due to ammonia. A sweetish smell of acetone is detected in ketosis.

Sediments

Fresh urine is clear, but keeping it for sometime, a flocculum separates occasionally. This consists of nucleoprotein, mucoprotein along with epithelial cells. In alkaline urine these sediments are added with precipitated calcium phosphate and ammonium magnesium phosphate, oxalate, and urate.

DISORDERD OF RENAL FUNCTION

There are many renal diseases which associated with abnormal renal function such as appearance in the urine of proteins, leukocytes, red cells, and casts. Other important consequences of renal disease are loss of the ability concentrate or dilute the urine, uremia, acidosis, and abnormal retention of sodium.

Proteinuria

Proteinuria is the presence of abnormal amounts of protein in the urine, particularly albumin (albuminuria). It produced by increased the permeability of the glomerular capillaries in association with many renal diseases. The amount of protein may be very large, and in nephrosis the urinary protein loss may exceed the rate at which the liver can synthesize plasma proteins. The resulting hypoproteinemia reduces the plasma osmotic pressure, which leads to (i) increase of the filtration force and hence reduction in the plasma volume, sometime to dangerously low levels; and (ii) accumulation of edema fluid in the tissues. There is a benign condition that causes proteinuria in which the protein appears in the urine when some individuals are in the standing position (orthostatic, postural, albuminuria), whereas when they are lying down their urine formed is protein-free.

Uremia

Uremia is produced by the accumulation in the blood of the breakdown products of protein metabolism. Elevated blood urea nitrogen (BUN) and creatinine provide an index of the severity of the uremia. Rise in plasma levels of BUN and creatinine depend on the fall in the glomerular infiltration rate. The

symptoms of uremia include lethargy, anorexia, nausea, and vomiting, mental deterioration and confusion, muscle twitching convulsion.

Plasma creatinine is not affected by diet because it produced endogenously by creatine breakdown. As circulating creatinine derived from metabolism of tissue creatine, its plasma levels would be expected to increase with increased tissue breakdown of creatine, but this is so less than production of urea. Therefore, creatinine would seem preferable to urea estimation as an index of renal function.

Acidosis

In chronic renal disease, acidosis can be developed from the failure of kidney to excrete the acid products of digestion and metabolism. In most cases of chronic renal disease the urine is maximally acidified, and acidosis develops because the total amount of H^+ that can be secreted is reduced because of impaired renal tubular production of NH_4^+. In rare syndrome of renal tubular acidosis, there is specific impairment of the ability to make the urine acidic, and other renal functions are usually normal.

Loss of concentrating and diluting ability

In renal disease, the urine becomes less concentrated and its volume is often increased, producing the symptoms of polyuria. The ability of the kidney to form a dilute urine is often retained, but in advanced renal disease, the osmolality of the urine becomes fixed at about that of plasma, indicating that the diluting and concentrating functions of the kidney have been lost. The loss of ability of the kidney to dilute and concentrate the urine depends on normal tubular function, as indicated by the counter-current multiplication and the presence of ADH, and the loss of functioning nephrons. When most of nephrons are destroyed, urine volume falls and oliguria or even anuria is present.

Abnormal sodium metabolism

In many patients of renal disease, excessive amounts of sodium are retained in the body and the patients become edematous. There are at least three causes of sodium retention in renal disease: (i) Acute glomerulonephritis: This disease affect primarily the glomeruli, therefore a marked decrease in the amount of sodium filtered is produced. (ii) Nephrotic syndrome: It is due to increased secretion of aldosterone which increases the retention of sodium into the body. In this condition, plasma protein level is decreased leading to movement of fluid from the plasma into the interstitial spaces and decline in the plasma volume. The latter stimulates the secretion of aldosterone via the renin-angiotensin system. (iii) Heart failure: This condition leads to sodium retention and edema in renal disease.

Anemia and secondary hyperparathyroidism

Anemia and secondary hyperparathyroidism represent also features of chronic renal failure, because impaired kidney does not synthesize erythropoietin and 1,25-dihydroxcholecalciferol, respectively.

THE HEMODIALYSIS

In case of renal failure the principle of dialysis is used to purify the blood of patients. The left portion of the Figure 24 ("Dialysis unit") shows the mechanism used today in artificial kidneys. Small molecules like urea are removed from the

blood because they are free to diffuse between the blood and the bath fluid, whereas large molecules (e.g., plasma proteins) and cells remain confined to the blood. The bath fluid must already have had essential salts added to it to prevent the dangerous loss of these ions from the blood. Note that blood and bath fluid flow in opposite directions across the dialysis membrane. This counter-current exchange maintains a diffusion gradient through the entire length of the system.
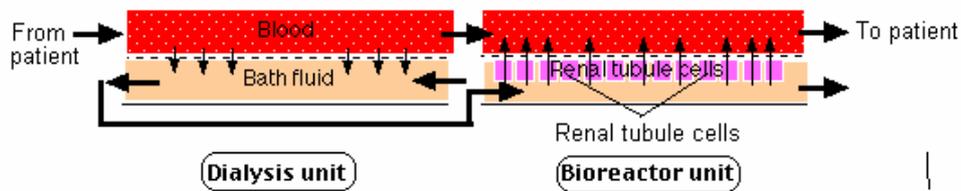

Figure 24. Scheme of the mechanism used in artificial hemodialysis. An anticoagulant is added to the blood so it will not clot while passing through the machine. The anticoagulant is neutralized as the blood is returned to the patient.

Artificial kidneys have proved of great benefit in helping patients of acute kidney malfunction survive the crisis until their own kidneys resume operation. They have also enabled people suffering from chronic kidney failure to remain alive, though at an enormous expense of time (often three sessions of 6 or more hours per week), money, and psychological well-being. Furthermore, although dialysis does a good job at removing wastes, it cannot perform the other functions of the kidney: (1) Providing precise homeostatic control over the concentration of such vital ingredients as glucose and Na^+. (2) Secreting its hormones

Hemodialysis in the future

In an attempt to solve these problems, a research team at the University of Michigan is experimenting with adding a "Bioreactor unit" to the dialysis unit. The bioreactor consists of many hollow, porous tubes on the inner wall of which is attached a monolayer of proximal tubule cells (derived from pigs). The dialysis bath fluid passes through the lumen of the tubes where molecules and ions can be picked up by the apical surface of the cells. Discharge of essential molecules and ions (as well as hormones) at the basolateral surface of the cells places these materials back in the blood (just as the proximal tubule cells in the nephron normally do). So far, all the testing has been done using dogs, but the results seem promising.