Lecture # 39

Mechanism of Urine Formation

Objectives:
1. Define the basic renal processes that result in urine formation.
2. Understand the renal handling of different substances and how does this determine the urinary excretion of these substances.
3. Understand how glomerular filtrate is formed and the composition of this filtrate.
4. Describe main determinants of solute filterability.
5. Define glomerular filtration rate (GFR) and forces affecting its formation.
6. Define renal blood flow (RBF), renal plasma flow (RPF) and the filtration fraction (FF).
7. Describe the different mechanisms that regulate RBF and GFR:
   a. Auto regulation.
   b. Nervous regulation.
   c. Hormonal regulation.

Reference book: Kaplan USMLE step 1Lecture note 2021 (pages # 203-213)

BASIC MECHANISM OF URINE FORMATION:

Urine is formed as a result of *filtration of plasma* in glomeruli (=glomerular filtration) and *reabsorption, secretion* and synthesis processes in renal tubules.

1. **GLOMERULAR FILTERATION (GF):** Urine formation begins with glomerular filtration, the bulk flow of *protein-free plasma* from the glomerular capillaries through glomerular membranes into Bowman's space. This fluid is called ***glomerular filtrate***. Normally glomerular capillary bed received about 650 ml plasma/ minute, of which only about 1/5 (**125 ml**) is **filtered in Bowman's capsule** while remaining **4/5 (525 ml) pass to peritubular capillaries**. GF is ***non-selective*** i.e. both useful & waste materials are filtrated. It is a ***passive process***. GF is called ***primary urine***.

Materials Filtered

The following are **easily or freely filtered**:

- Major electrolytes: sodium, chloride, potassium, bicarbonate
- Metabolic waste products: urea, creatinine
- Metabolites: glucose, amino acids, organic acids (ketone bodies)
- Non-natural substances: inulin, PAH (p-aminohippuric acid)

• Lower-weight proteins and peptides: insulin, myoglobin

The following are **not freely filtered**:

• Albumin and other plasma proteins

• Lipid-soluble substances transported in the plasma attached to proteins, such as lipid-soluble bilirubin, T4 (thyroxin), other lipid-soluble hormones; unbound lipid-soluble substances such as free-cortisol are filtered and can appear in the urine As blood flows through the glomerular capillary, plasma is filtered, but albumin is not, so the plasma albumin concentration and oncotic pressure increase.

2. **TUBULAR PROCESSES:** in renal tubules, about 124 ml/min of urine are reabsorbed back into peritubular capillaries (wanted substance) and more of unwanted substances (about 1 ml/min) are secreted from peritubular capillaries into tubules forming the actual urine. These processes are:

a. ***Tubular reabsorption:*** It is the process of transfer of solutes and water *from tubular lumen to peritubular capillary plasma*.

b. ***Tubular secretion:*** It is transfer of solutes from *peritubular capillary plasma to tubular lumen* (opposite direction of reabsorption) or by ***synthesized*** *in renal tubular cell*. For example ammonia is synthesized from glutamine amino acids and is secreted into tubular fluid.

c. The rates at which the different substances are excreted in urine represent the sum of the renal processes. Expressed mathematically:

Urinary excretion rate = (filtration rate – reabsorption rate) + secretion rate.

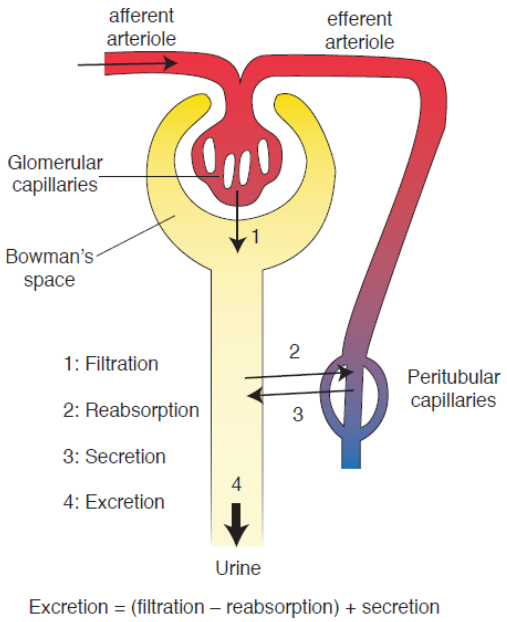

Figure 39. 1: Basic renal processes.

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CHARACTERISTICS & COMPOSITE OF GLOMERULAR FILTRATE:

The glomerular filtrate has **same characteristics of plasma**. It is **pH is 7.4**, **specific gravity 1010** and **osmolality 300 mOsm/litre**. Glomerular filtrate rate (GFR) is proportionate with body surface area. GFR in *more* in males than females and *decreased* by age (only 60% at 70 years old).

The ***filtered fluid*** is essentially **protein –free and devoid of cellular elements**. The concentrations of other constituents of plasma, including *salts and organic molecules* that are *not bound to plasma proteins*, as glucose and amino acids, are similar in the plasma and glomerular filtrate.

DETERMINANTS OF SOLUTE FILTERABILITY:

Despite the high filtration rate, glomerular filtration barrier is selective in determining which molecules will filter, based on *their size and electrical charge*.

1. ***Molecular size:*** the glomerular membranes provide no restriction to movement of molecules with *molecular weights* **less** than **7000 daltons** (**molecular diameter** of **4 nm** or 40 Å) and almost **total prevent** passage of molecules with **molecular weights** of **about 70,000 daltons**

(molecular **diameter of 8 nm** or 80 Å). **For molecules** between **7000 and 70,000 daltons**, **filterability becomes progressively smaller as molecule becomes bigger**.

2. ***Electrical charge of molecule***: in general, **negatively charged large molecules** are filtered **less easily than positively charged molecules of equal molecular size**. Albumin (molecular weight of 69,000 daltons) is restricted from filtration because of its negative charge.

GLOMERULAR FILTRATION RATE (GFR):

Definition: ***The volume of plasma filtrate by both kidneys per unit time*** is known as **GFR**. The average volume filtered into Bowman's capsule is *180 liters per day*. Since the total volume of plasma in cardiovascular system is about 3 liters, this means that all plasma volume is filtered about *60 time's plasma volume*. This is important to enable the kidneys to regulate the constituents of the internal environment and to excrete large quantities of waste products.

DIRECT DETERMINATION OF GFR:

The rate of fluid movement, by filtration, in any of the body's capillaries is determined by the *permeability of the capillaries*, *their surface area*, and the *net filtration pressure (NFP)* acting across them.

Glomerular filtration rate= capillary permeability X surface area X net filtration pressure (NFP)

Because it is difficult to estimate surface area of a capillary bed, a parameter called ***filtration coefficient*** (K_f) is used to denote ***product of capillary permeability and surface area***. Surface area of glomerular membrane = $0.8m^2$. The rate of glomerular filtration is considerably greater in glomerular capillaries versus systemic capillaries mainly because the K_f is much higher. **Filtration coefficient** is the *amount of glomerular filtrate per one mmHg*. The ***NFP*** represents sum of hydrostatic and colloid osmotic forces (Starling forces) that either favors or opposes filtration across glomerular capillaries.

Therefore, **GFR = K_f x NFP**

K_f = GFR / NFP = 125/10= 12.5 ml/min/mmHg

FORCES AFFECTING GLOMERULAR FILTRATION:

FILTERATION FORCES:

1. ***Glomerular capillary hydrostatic pressure (P_{GC}):*** The pressure of blood in the glomerular capillaries averages ***60 mmHg***. This is higher than other capillaries of the body because the afferent arterioles, having larger diameters, offer less resistance than most arterioles and so more of the arterial pressure is transmitted to the capillaries. In addition, efferent arterioles being smaller than afferent arterioles offer post-capillary resistance.

2. ***Colloid pressure in Bowman's capsule:*** it is practically ***zero*** as only very little amount of protein is normally filtered in capsular fluid.

FORCES OPPOSING FILTRATION:

1. Hydrostatic pressure in Bowman's capsule (P_{BC}): The fluid in Bowman's capsule exerts a hydrostatic pressure of ***18 mmHg***.

2. Colloid osmotic pressure of glomerular capillary plasma proteins (π_{GC}): It results from presence of protein in glomerular capillary plasma. It is about ***32 mmHg***. This is slightly higher than across other capillaries (28 mmHg) because more water is filtered out of glomerular blood, so concentration of plasma proteins is higher than elsewhere. As blood passes from afferent arterioles through glomerular capillaries, colloid osmotic pressure rises to 36 mmHg by time the blood reaches efferent end of capillaries (average of 32 mmHg).

Therefore, **net glomerular filtration pressure**, the algebraic sum of the four forces, is about **10 mmHg**. This pressure starts urine formation, by forcing a protein free filtrate of plasma through glomerular membranes into Bowman's capsule and then down tubule into the renal pelvis.

GFR = K_f x NFP

NFP = P_{GC} – (P_{BC} + π_{GC}) = 60 – (18 + 32) = 10 mmHg.

FACTORS INFLUENCNG GFR:

1. Glomerular capillary hydrostatic pressure (P_{GC}): **P_{GC} is affected in three ways**:

a. ***Changes in renal arteriolar pressure:*** **an increase in blood pressure** above 180 mmHg tends to **increase P_{GC}** and hence **increase in GFR, decrease in ABP below 80 mmHg → decrease GFR**. However, as will discuss later, ***between 80-180 mmHg,*** GFR is not changed because of **autoregulatory mechanisms**.

b. ***Changes in afferent arteriolar resistance:*** **afferent arterioles vasoconstriction reduces P_{GC}** and **decreases GFR**.

c. ***Changes in efferent arteriolar resistance:*** **increased resistance of efferent arteriole → increases P_{GC}** and **raises GFR**.

2. **Bowman's capsule hydrostatic pressure (P_{BC})**: **P_{BC}** can **increase** markedly in certain pathological states associated with obstruction of urinary tract (e.g. a stone ureter), causing a **decrease in GFR**.

3. Glomerular capillary colloid osmotic pressure (π_{GC}): An **inverse relationship** exists **between π_{GC} and GFR**. An **increase in plasma protein concentration** (as in dehydration) tends to **increase π_{GC}** and hence **reduces GFR**. Decreased protein synthesis (e.g. liver diseases) or protein loss in urine lead to a decrease in plasma protein concentration and thus π_{GC}.

4. Filtration coefficient (K_f): Changes in K_f can be caused by glomerular disease and drugs. It is also under physiological control. ***Both factors on which K_f depends*** - **surface area** and **permeability of glomerular membrane** - can be modified by contractile activity within membrane.

a. Surface area: Glomerular capillaries are held together by **mesangial cells**. **Contraction of these mesangial cells closes off** a

portion of **filtering capillaries** thereby **reducing surface area** and **GFR**.

b. Permeability: **Podocytes** possess actin-like contractile filaments whose contraction or relaxation can decrease or increase number of filtration slits, which determines permeability; **more open slits, greater permeability**.

RENAL BLOOD FLOW (RBF) AND ITS PHYSIOLOGICAL REGULATION

In a resting adult, the combined blood flow through both kidneys is about **1200 ml/min** (*20% of cardiac output*). As with other organs, blood flow supplies the kidneys with nutrients and removes waste products. However, the high flow to kidneys greatly exceeds this need. The purpose of this additional flow is to supply enough plasma for the high rates of glomerular filtration that are necessary for precise regulation of body fluid volumes and solute concentrations. **Renal fraction** is defined as **portion of cardiac output that passes through kidneys**. Normally it **average 20%**, **ranging from (12-30%).**

- Given a normal hematocrit of 0.45, the renal plasma flow (**RPF**) = 0.55 x 1.2 L/min = **650 ml/min.** Since GFR = 125 ml/min thus, of 650 ml/min of plasma that enters glomeruli, 125/650 or **20%**, filters into Bowman's capsule, the remaining plasma pass through efferent arteriole into peritubular capillaries. This ratio - **GFR/RPF**- is known as the **filtration fraction (FF). So filtration fraction is defined as fraction of plasma that filters through glomerular membrane**.

REGIONAL BLOOD FLOW & OXYGEN CONSUMPTION:

- **Renal cortex: receives great majority of renal blood flow and little oxygen** is extracted from the blood, as its main function is filtration.
- **Renal medulla:** its **blood flow is sluggish** and accounts for **1-2% of total RBF** because of high resistance offered by vasa recta. Because of

metabolic work done in medulla (mainly active reabsorption of sodium), **oxygen consumption is large**. This makes the medulla prone to hypoxia if flow is reduced further. Local vasodilator agents such as nitric oxide and prostaglandins, function in this region to maintain balance between low blood flow and metabolic needs.

Blood flow (F) is determined by mean arterial pressure (MAP) and resistance (R) of renal arterioles (F=ΔMAP/R).

Flow (F) = pressure gradient (ΔMAP, mean arterial blood pressure) **/ resistance (R)**

Independent response of the afferent and efferent arterioles

Thee table below illustrates the expected consequences of independent isolated constrictions or dilations of the afferent and efferent arterioles.

Table 39. 1: Consequences of Independent Isolated Constrictions or Dilations of the Afferent and Efferent Arterioles

REGULATION OF RENAL BLOOD FLOW (RBF) & GFR:

- **RBF** is **determined** largely by **pressure gradient across the renal vasculature (by difference between renal artery and renal vein hydrostatic pressures), divided by total renal vascular resistance.**

RBF = (renal artery pressure – renal vein pressure)/ total renal vascular resistance.

- **Renal artery pressure** is about **equal to systemic arterial pressure** and **renal venous pressure** average **about 3-4 mmHg** under most conditions. As in other vascular beds, the **total vascular resistance** through the kidneys is **determined** by the **sum of the vascular resistance in the individual vasculature segments**, including the arteries, arterioles, capillaries, and veins. **Most** of the **renal vascular resistance resides in three major segments**: **interlobular arteries**, **afferent arterioles, and efferent arterioles**.

- RBF is regulated by both **intrinsic and extrinsic controls**. Intrinsic controls (renal autoregulation) act locally within the kidney, while extrinsic controls by the nervous and endocrine systems.

a. <u>AUTOREGULATION (INTRINSIC) MECHANISMS:</u>

Autoregulation is **intrinsic mechanism** in the kidney that keeps the GFR & RPF nearly constant despite changes in ABP between **(80-180 mmHg).** Autoregulation **occurs in denervated (e.g. transplanted) and isolated, perfuse kidneys (**not dependent on the nerve supply or on blood-borne substances**).** It is achieved by *changes in renal vascular resistance* - primarily through afferent arterioles of kidneys by two mechanisms:

1. *MYOGENIC MRCHANISM:* Vascular smooth muscle **responds with constriction to an increase in stretch** and **with relaxation** to **decrease in stretch**. Its **cellular mechanisms** involve **both stretch-activated cation (Ca^{++}) channels** and **release of endothelial vasoactive factors**.

Accordingly, when arterial pressure raises → stretch of afferent arterioles → increase in calcium influx from extracellular fluid into muscle fiber → direct vasoconstriction → prevent increase in RBF.

When arterial pressure fall → relaxation of afferent arterioles → direct vasodilatation → prevent decrease in RBF.

2. *TUBULOGLOMERULAR FEEDBACK:*

Release of chemical vasoactive substances from renal tubules that acts on afferent and efferent arterioles. Several chemicals have been identified (**vasoconstrictors** e.g. adenosine, ATP, endothelin, thromboxane A_2 and **vasodilators** e.g. prostaglandin, bradykinin).

- Increase in arterial blood pressure (ABP) → increase in RBF & GFR → increase in rates of fluid flow through loop of Henle and first part of DCT, including macula densa → release of vasoconstrictor chemical by juxtaglomerular apparatus →

vasoconstriction of afferent arterioles → decrease RBF & GFR to normal.

- Decrease in ABP → decrease in RBF & GFR → increase reabsorption of sodium & chloride in ascending loop of Henle → decrease delivery of NaCL to macula densa → signals that cause vasodilatation of afferent arterioles & vasoconstriction of efferent arterioles (renin release) → increase RBF & GFR to normal.

Importance of autoregulation:

1) **It GFR is much increased;** the tubular fluid would pass rapidly in the tubules allowing ***minimal reabsorption of essential*** substances which will be lost in urine.

2) If **GFR was too low**, **kidneys *would not* be able to eliminate sufficient quantities of wastes**, excess electrolytes & other materials that should be excreted.

b. **NERVOUS CONTROL:**

- Strong activation of renal **sympathetic fibers** activity causes **vasoconstriction** especially of afferent arterioles which leads to **decrease renal blood flow** and also increased activity of granule cell beta 1 adrenoreceptors that leads to increase in renin secretion that leads to increase plasma angiotensin II and increase of decreased arterial blood pressure. The renal sympathetic nerves seems to be important in reducing GFR during severe acute disturbance, lasting for few minutes to few hours, such as those elicited by severe hemorrhage.
- **Mild and moderate sympathetic stimulation** has little influence on renal blood flow and GFR.
- **In normal resting person,** there appears to be **little sympathetic tone to kidney.**

c. HORMONAL AND PARACRINE CONTROL:

a. **VASOCONSTRICTORS:**

*1. **Angiotensin II:***

Secretion of the enzyme, **renin from** storage granules in **juxtaglomerular** cells of the **afferent arteriole** is **increased by three mechanisms**:

(1) **Diminished stretch of the afferent arteriolar wall**.

(2) **Low sodium diet**.

(3) **Adrenoreceptor activation** by sympathetic nerves supplies JGA.

The principal **action of renin** is to convert freely circulating substrate **angiotensinogen** into **angiotensin I** (biologically inactive) then under the influence of angiotensin **converting enzyme** (secreted by the liver and lung), **converted** to **angiotensin II**.

2. ***Norepinephrine and epinephrine:*** **secreted** by **renal medulla**, cause **vasoconstriction** by binding to alpha adrenergic receptors that located on **afferent arterioles** and thereby **decrease RBF & GFR**.

3. *Antidiuretic hormone (vasopressin).*

4. *Glucocorticoids.*

5. *Endothelin:* **secreted by damage vascular endothelial cells** of renal vessels and other tissues, causes **profound vasoconstriction of afferent and efferent arterioles** and **decreases GFR and RBF**.

6. *Adenosine:* produced within the kidneys, causes **vasoconstriction of afferent arteriole** *(in contrast to its vasodilator effect on most vascular beds),* thereby **reducing RBF and GFR**. It may play a role in tubuloglomerular feedback**.**

7. *Thromboxane A2, B2.*

8. *Leukotriene D4, C4.*

9. *Platelet-activating factor.*

b. <u>VASODILATRS</u>:

1 . ***Dopamine:*** made in kidney in small amount.

2. ***Atrial natriuretic peptide (ANP):*** secretion by heart **increases** when atria of the heart stretch due to increase blood volume. ANP is a **vasodilator and increases sodium excretion** through a number of mechanisms, including increase in GFR.

3. ***Nitric oxide:*** an endothelium-derived factor that causes **vasodilation** of afferent & efferent arterioles.

4. ***Prostacyclin and prostaglandin E_2 (prostaglandins):*** may not regulate RBF and GFR in healthy people but during pathological conditions such as hemorrhage, they are **locally** produced within kidneys.

5. ***Bradykinin:*** produced in kidney.

6. ***Acetylcholine***.