# URINE FORMATION FUNCTION OF KIDNEY. PHYSICAL-CHEMICAL PROPERTIES AND CHEMICAL COMPOSITION OF NORMAL. PATHOBIOCHEMISTRY OF KIDNEYS. PATHOLOGICAL COMPONENTS OF URINE

Kidney functions in organism:

a) excretion of final metabolic products;

b) maintaining of acid-base balance;

c) water-salts balance regulation;

d) endocrine function.

http://www.youtube.com/watch?v=6x5pVoMb_vI&feature=related

**Physical and chemical characteristics and components of urine:**

a) volume, physical and chemical properties of urine;

b) inorganic components of urine;

c) organic components of urine.

***Key words and phrases:***

Kidney – the couple organ, which is responsible for excriting of final products of metabolism and for homeostasis. They regulate water and mineral metabolism, acid-base balance, excriting of nitrogenous slags, osmotic pressure. Also they regulate arterial pressure and erhythropoesis.

http://www.youtube.com/watch?v=glu0dzK4dbU

Nephron – is the structural and functional unit of kidney.

Urine – fluid with different organic and inorganic compouds, which must be excreted (excess of water, final products of nitrogen metabolism, xenobiotics, products of protein's decay, hormones, vitamins and their derivates). Most of them

present in urine in a bigger amount than in blood plasma. So, urine formation – is not passive process (filtration and diffusion only).

In basis of urine formation lay 3 processes: filtration, reabsorbtion and secretion.

**Glomerulal filtration.** Water and low weight molecules go to the urine with help of following powers: blood hydrostatic pressure in glomerulas (near 70 mm Hg), oncotic pressure of blood plasma proteins (near 30 mm Hg) and hydrostatic pressure of plasma ultrafiltrate in glomerulal capsule (near 20 mm Hg). In normal conditions, as You see, effective filtration pressure is about 20 mm Hg.

Hydrostatic pressure depends from correlation between opening of a. afference and a. efference.

Primary urine formed in result of filtration (about 200 L per day). Between all blood plasma substances only proteins don't present in a primary urine. Most of these substances are undergone to the following reabsorbtion. Only urea, uric acid, creatinin, and other final products of different metabolic pathways aren't undergone to the reabsorbtion.

For evaluate of filtration used **clearance** (clearance for some substance – it is a amount of blood plasma in ml, which is cleaned from this substance after 1 minute passing through kidney).

Drugs which stimulate blood circulation in kidney (theophyllin), also stimulate filtration. Inflammatory processes of renal tissue (nephritis) reduce filtration, and azotaemia occurred (accumulation of urea, uric acid, creatinin, and other metabolic final products).

**Reabsorbtion.** Lenght of renal tubules is about 100 km. So, all important for our organism are reabsorbed during passing these tubules. Epitelium of renal tubules reabsorb per day 179 L of water, 1 kg of NaCl, 500 g of $NaHCO_3$, 250 g of glucose, 100 g of free amino acids.

All substances can be divided into 3 group:

1. Actively reabsorbed substances.
2. Substances, which are reabsorbed in a little amount.

3. Non-reabsorbed substances.

To the first group belong $Na^+$, $Cl^-$, $Mg^{2+}$, $Ca^{2+}$, $H_2O$, glucose and other monosaccharides, amino acids, inorganic phosphates, hydrocarbonates, low-weight proteins, etc.

Na+ reabsorbed by active transport to the epitelium cell, then – into the extracellular matrix. $Cl^-$ and $HCO_3^-$ following $Na^+$ according to the electroneutrality principle, water – according to the osmotic gradient. From extracellular matrix substances go to the blood vessels. $Mg^{2+}$ and $Ca^{2+}$ are reabsorbed with help of special transport ATPases. Glucose and amino acids use the energy of $Na^+$ gradient and special carriers. Proteins are reabsorbed by endocytosis.

Urea and uric acid are little reabsorbable substances.

Creatinin, mannitol, inulin and some other substances are non-reabsorbable.

Henle's loop play important role in the reabsobtion process. Its descendent and ascendent parts create anti-stream system, which has big capacity for urine concentration and dilution. Fluid which passes from proximal part of renal tubule to the descendent part of Henle's loop, where concentration of osmotic active substances higher than in kidney cortex. This concentration is due to activity of thick ascendent part of Henle's loop, which is non-penetrated for water and which cells transport $Na^+$ and $Cl^-$ into the interstitium. Wall of descendent part is penetrated for water and here water pass into the interstitium by osmotic gradient but osmotic active substances stay in the tubule. Ascendent part continue to reabsorb salt hypertonically, even in the absence of aldosteron, so that fluid entering the distal tubule still has a much lower osmolality than does interstitial fluid.

Some substances ($K^+$, ammonia and other) are **secreted** into urine in the distal part of tubules. $K^+$ is changed to $Na^+$ by the activity of $Na^+$-$K^+$ATPase.

# Summary of Stages of Urine Formation

**http://www.youtube.com/watch?v=aQZaNXNroVY&feature=related**

## I). Summary Stages of filtration

**A). Glomerular filtration:** small molecules enter tubule formed elements are too big

**B). Tubular reabsorption:** Molecules are reabsorbed into the blood stream.

From the nephron into the capillary network.

i.e. Glucose is actively reabsorbed by being transported on carriers. If the carriers are overwhelmed glucose appears in the urine indicating diabetes.

**C). Tubular secretion:** Substances are actively added to the tubular fluid from the capillaries to the tube

## II). Mechanisms of Urine Formation

**A). Glomerular filtration:**

hydrostatic pressure or fluid pressure gradient

osmosis

**B). Tubular reabsorption:**

diffusion

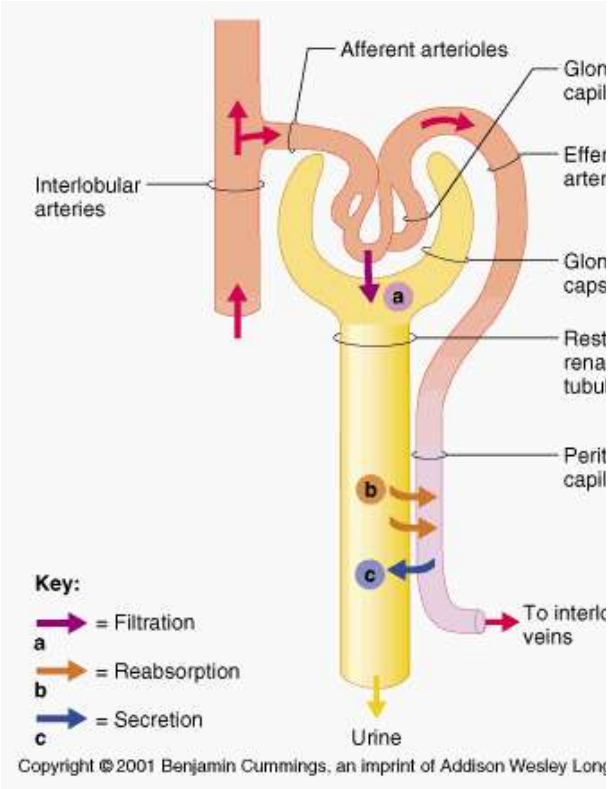
facilitated diffusion

osmosis

active transport

**C). Tubular secretion:**

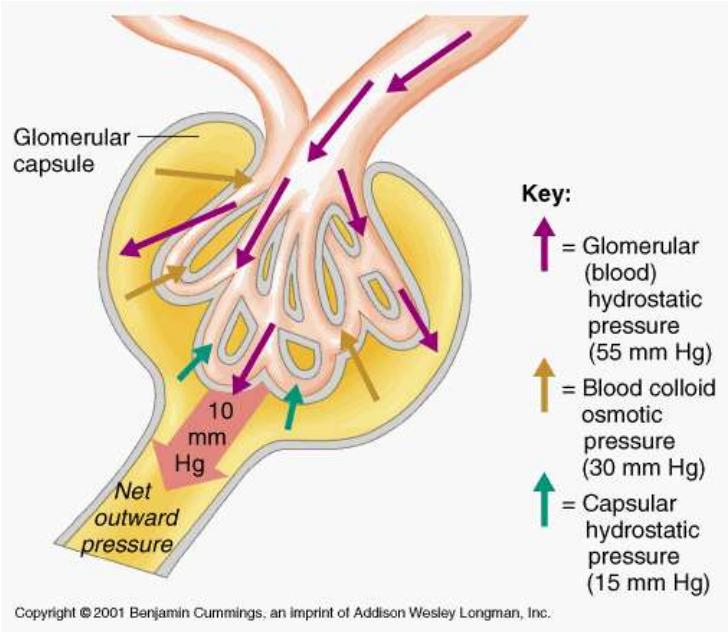
active transport




III**). Glomerular Filtration**

**Passive** and **nonselective**

Driven **hydrostatic pressure** form **the glomerular capillaries** and the **glomerular capsule**.




A**). Filtration membrane**

B). **Net Filtration Pressure**

(pressure going in) –(pressure pushing out)

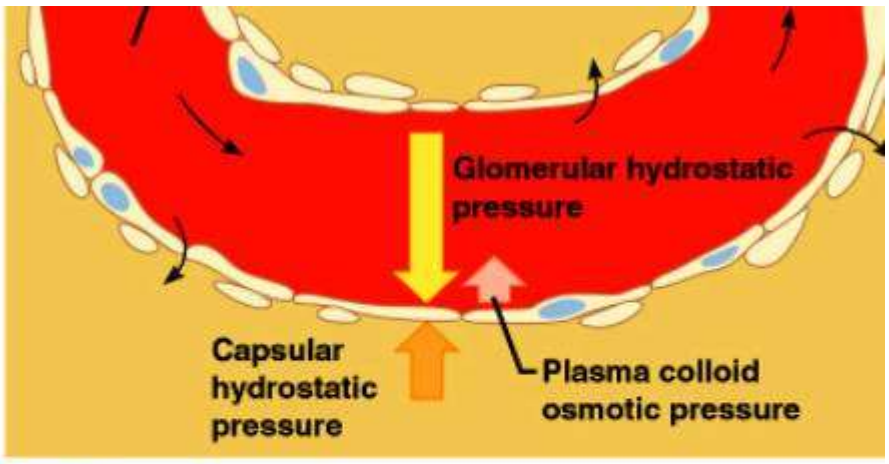
**Glomerular Hydrostatic Pressure:** Pressure pushing out of the glomerular capillaries

**Colloid Osmotic Pressure:** Pressure pushing into the glomerular capillaries because of differences in protein concentrations

**Capsular Hydrostatic Pressure**: Pressure pushing into the glomerular capillaries from fluid already in Bowman's capsule.

Glomerular Hydrostatic Pressure- (Colloid Osmotic Pressure +Capsular Hydrostatic Pressure)

(force favoring filtration)-(force opposing filtration)




| Net Outward Pressure | | |
|---|---|---|
| Outward force, glomerular hydrostatic pressure | = | +60 m |
| Inward force of plasma colloid osmotic pressure | = | −32 m |
| Inward force of capsular hydrostatic pressure | = | −18 m |
| Net outward pressure | = | +10 m |

C**). Glomerular Filtration Rate (GFR)**

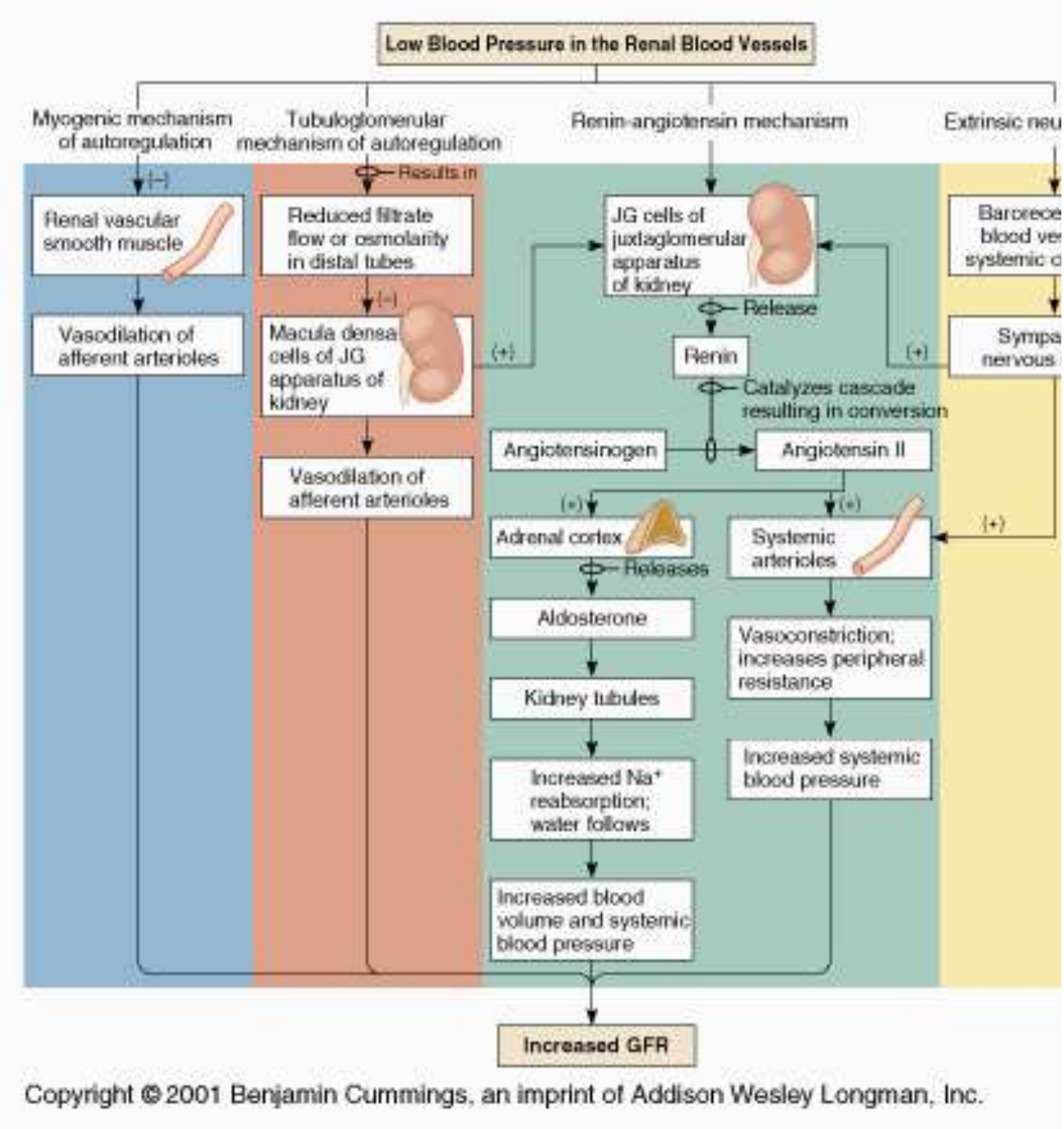
**http://www.youtube.com/watch?v=lH9IXpp5zTU**

1). surface area

2). permeability

3). Net Filtration Pressure

D**). Control of glomerular filtration**




1**). Intrinsic Controls**: Control from the renal system

**regulating the diameter of the afferent arteriole.**

**i). Myogenic Mechanism**

ii). **Tubuloglomerular feedback mechanism**

The **osmoreceptors** in distal tubules respond to **slowing flowing filtrate** (thus **decreased filtrate concentration**) by releasing **vasodilators** to the afferent arterioles.

In response to **fast filtrate rate** and thus **high solute concentration** by releasing **vasoconstrictors.**

**Large diameter →**

**Increase volume→**

**Results in a high net pressure →**

**And a fast GFR (rate) →**

**so**

**there is <u>no time</u> to reabsorb →**

**Results in a <u>high solute</u> concentration**

**& high osmotic pressure at the juxtaglomerular apparatus →so vasoconstrictor will be released**

**Small diameter →**

**lowers volume →**

**Results in a low net pressure →**

**And a slow GFR (rate) →**

**so**

**there is <u>too much time</u> to reabsorb →**

**Results in a <u>low solute</u> concentration →**

**& low osmotic pressure at the juxtaglomerular apparatus →**

**so**

**vasodilator will be released**

**iii). Renin-Angiotensin mechanism**

**Osmoreceptors** release **renin**,

Renin acts on **angiotensinogen,**

**angiotensin I**

**angiotensin II**

**angiotensin II** is a vasoconstrictor

**blood pressure in the entire body to rise**

**release aldosterone**

**aldosterone** increases reabsorption  Na+

blood pressure increases

2). **Extrinsic controls** (outside the renal system)

Sympathetic nerve fibers

the **adrenal medulla**

**epinephrine**

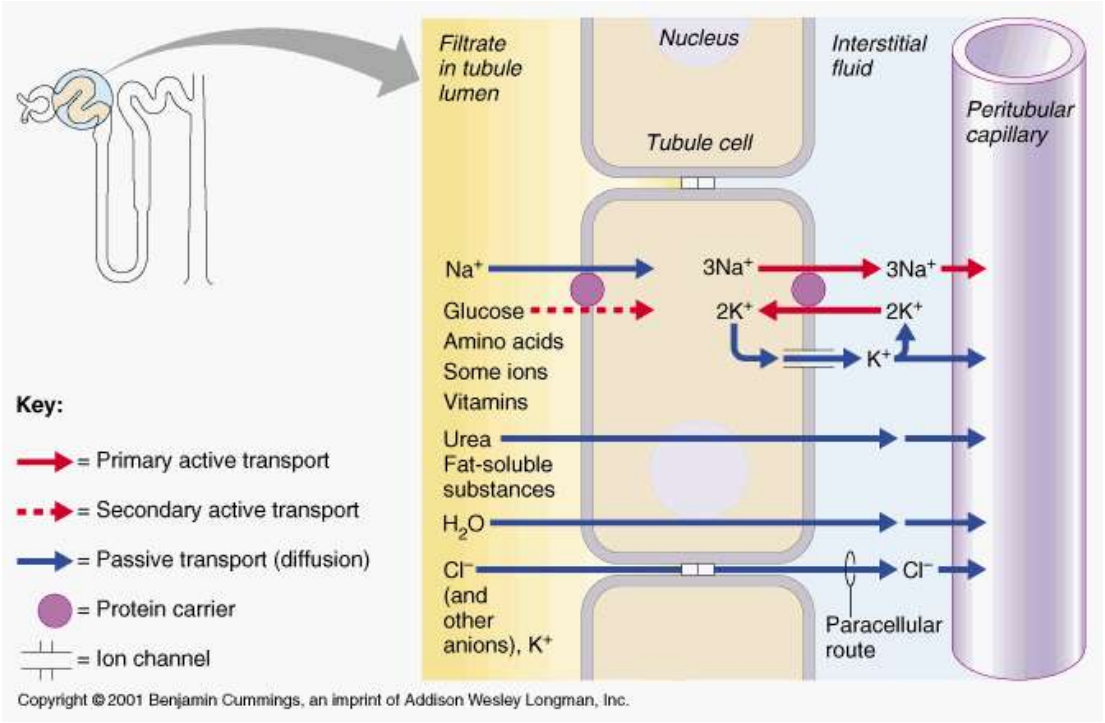
vasoconstriction in the **afferent arterioles**

inhibits **filtrate formation**.

IV). **Tubular Reabsorption**

Concentrating of the filtrate by returning solutes and water to the blood stream.

A). Methods of Reabsorption



1**). Active Tubular Reabsorption**

i). **Cotransport**

binding to the same as carrier complex

That creates a transport maximum **(Tm** mg/minute) for every solute.

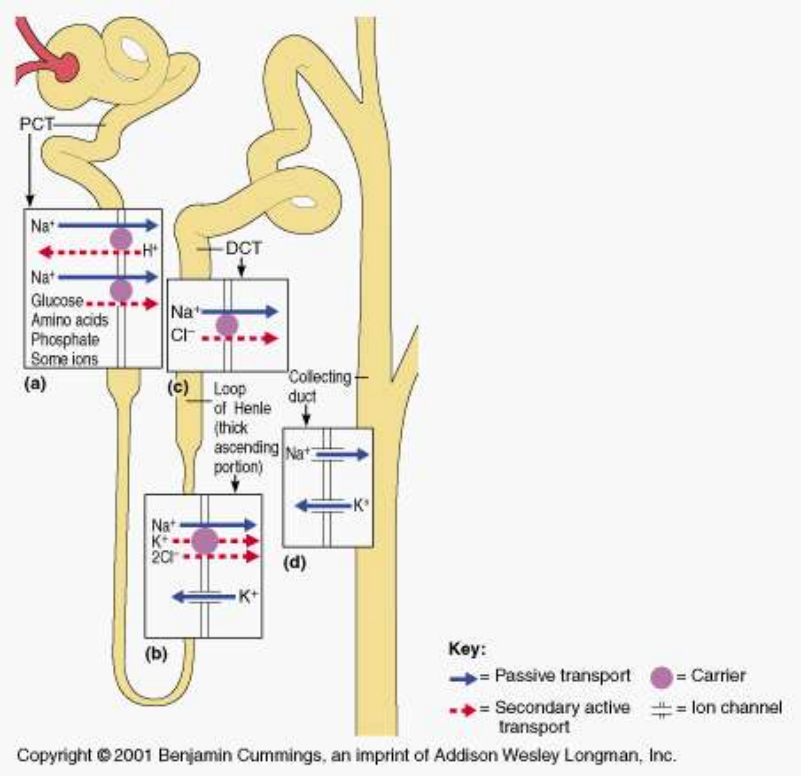
**When the carriers are exceeded the solute is excreted in the urine**.

( the Tm of glucose 375 mg/min glucose over this limit is excreted).

**ii). Pinocytosis**

2). **Passive Tubular Reabsorption**

http://www.youtube.com/watch?v=aQZaNXNroVY&feature=related



**i). tubular fluid through the epithelium**, into the **interstitial fluid** then diffuse into the **peritubular capillaries** and back into the **blood stream**.

ii). Substances **move across their electrochemical** gradient

**iii). Positively charged Na+ ions are actively transported**

a). **electrochemical gradient**

b). **osmotic gradient**

B). **Reabsorption in different sections of the tubule.**

**http://www.youtube.com/watch?v=KINOArtDeWg&feature=related**

1). **Glomerular capsule**:

2). **Proximal Convoluted Tubule**.

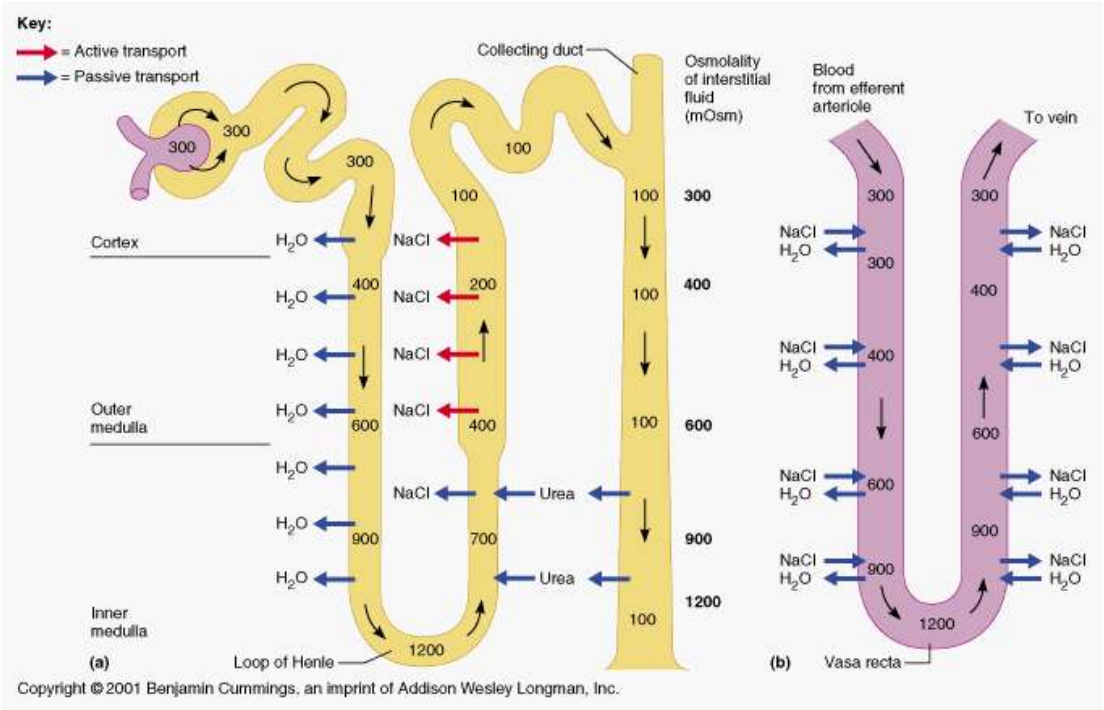
Na+ reabsorption
Water (water follows Na+)
glucose & amino acid
cations (+ ions)
anions (-ions)
Urea and lipid soluble solute
Proteins

3). **Loop of Henle**

**Permeability**

Water can leave **descending limb** but not the **ascending limb** of the loop of Henle

Na+ cannot leave **descending limb** but can leave the **ascending limb** of the loop of Henle





**Countercurrent Mechanism**

Maintains the high level of Na+ in the interstitial fluid

Fluid moving down the descending limb creates a current that is counter to the fluid moving up the ascending limb

Every time Na+ is actively removed from the ascending limb;

water in the descending limb is pulled out because of osmosis

So more Na+ that is actively removed the more water is pulled out.

**Mechanisms**

**Step a: Osmotic Gradient**

**Step b: Permeability to Solutes**

The **interstitial fluid becomes hypertonic**, but the **filtrate becomes hypotonic.**

(**filtrate loses salt it becomes increasingly dilute)**

**The loop creates a concentration gradient along it length.**

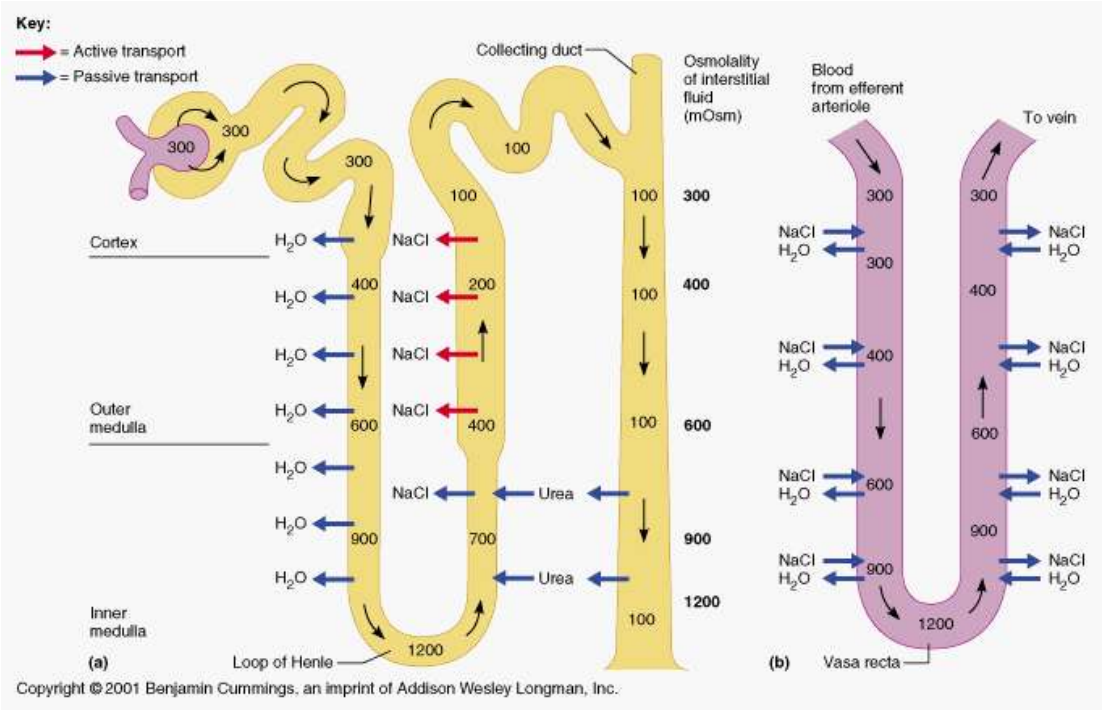
**(countercurrent multiplier.)**

This gradient is what causes the water to move out of the filtrate in the descending loop

**Step c. Function of the Vasa Rectus**

**Step d Collecting Duct**

Concentration of fluid:





4). **Distal Convoluted Tubule**

Water reabsorption here is dependent on hormones.

**Antidiuretic Hormone**.

**Aldosterone**.

5). **Collecting Duct**

V). **Tubular Secretion**

Reabsorption in reverse.

H+

K+

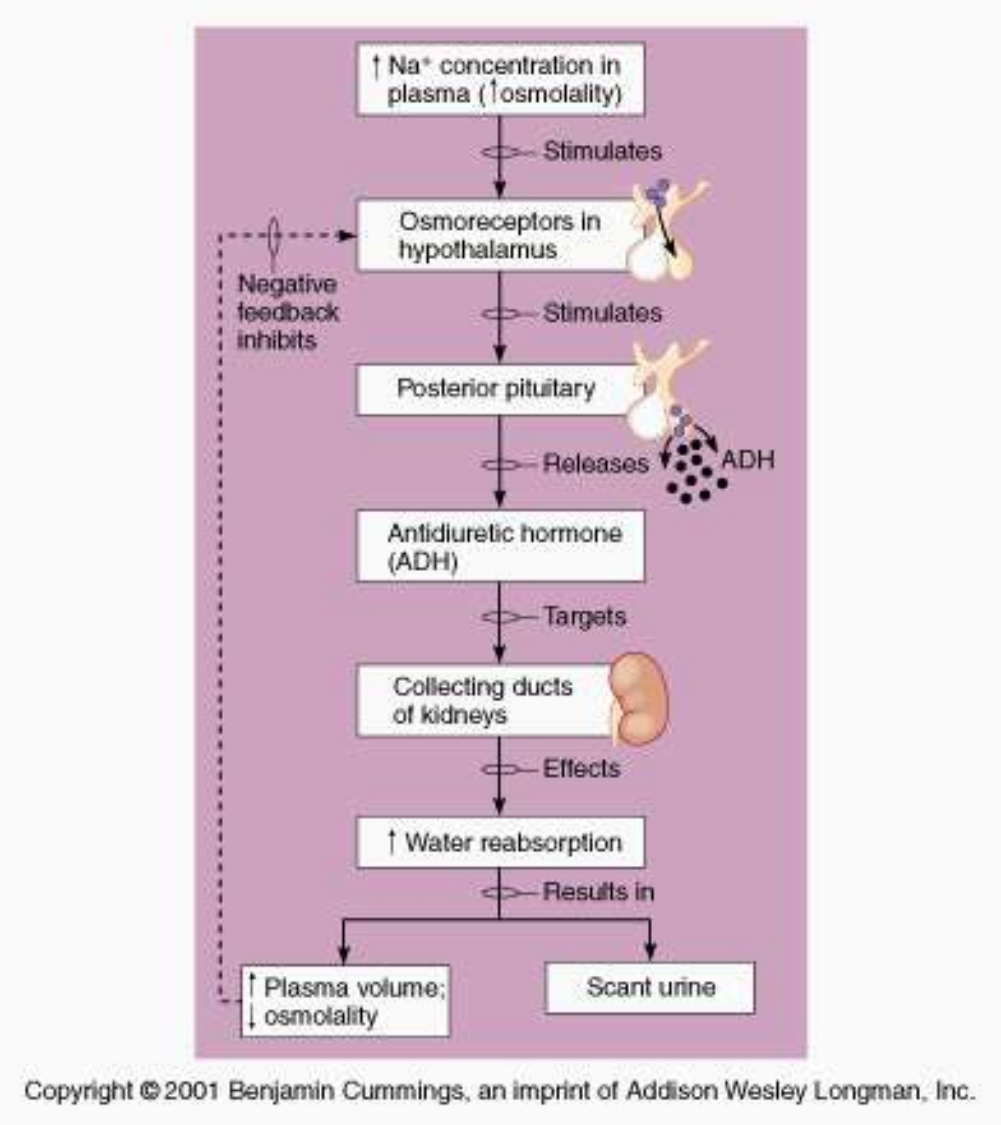
creatinine

ammonium ions

various drugs

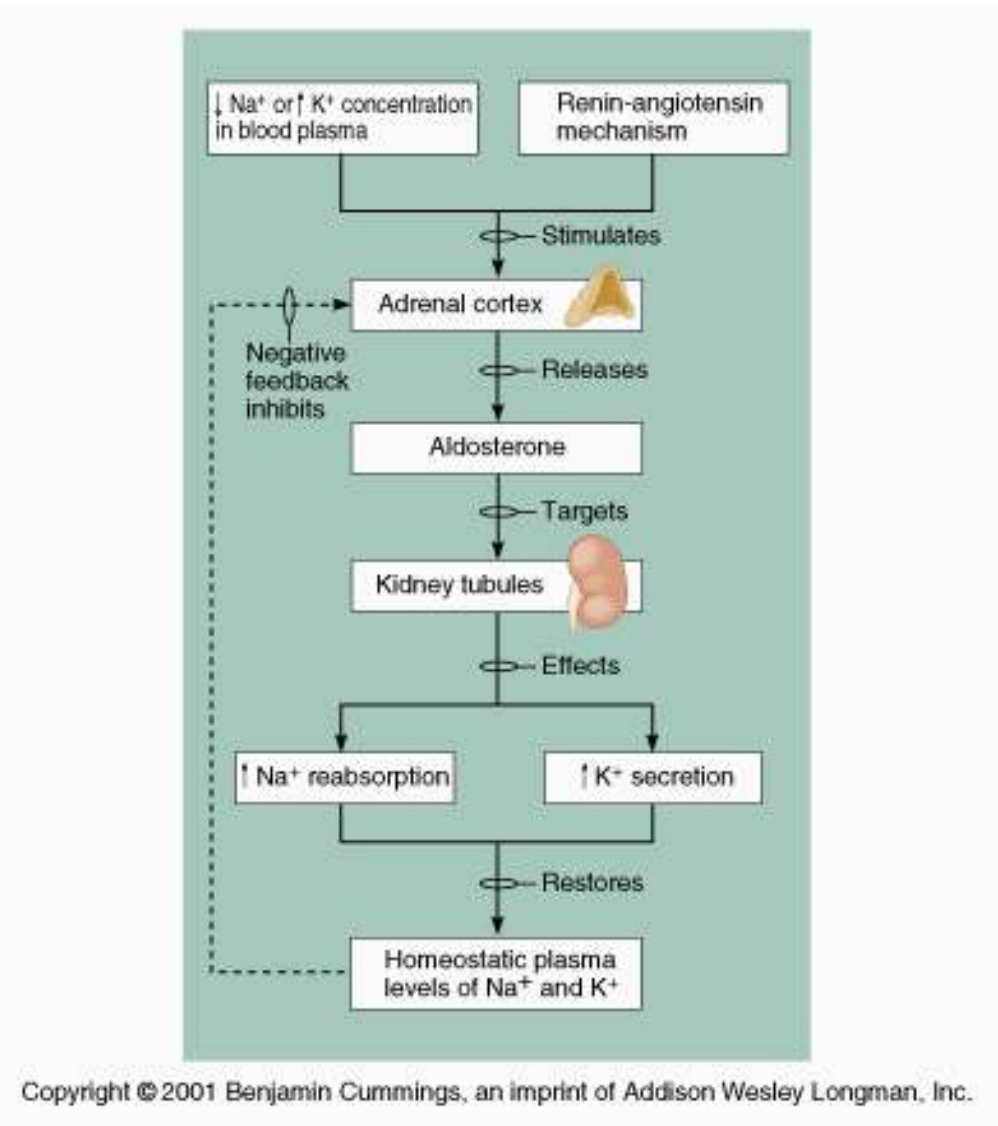
VI). **Hormones affecting renal function**

A). **ADH:**



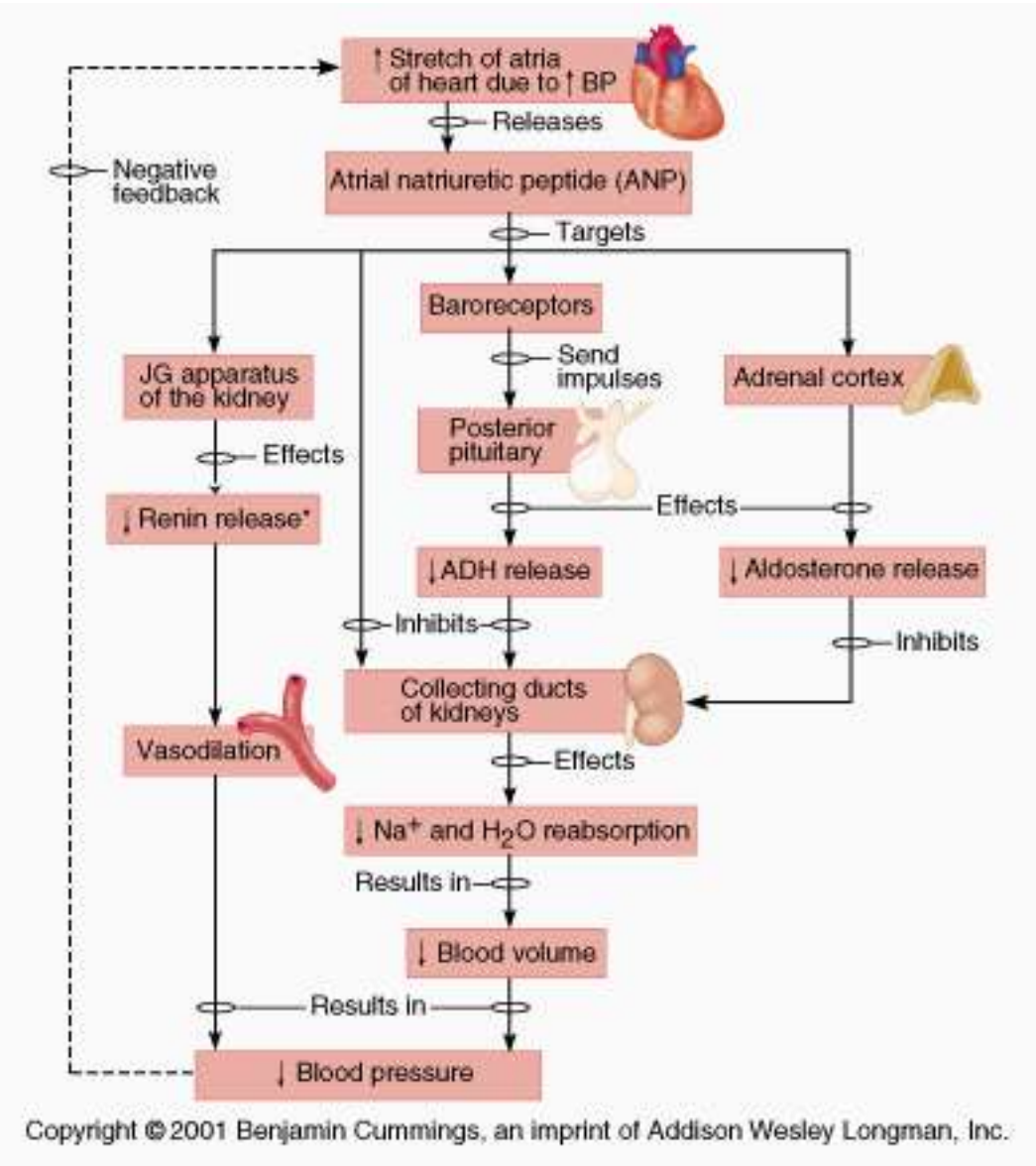


B). **Aldosterone:**





## C). Atrial Natriuretic Peptide




***Peculiarities of biochemical processes in kidney.***

Kidney have a very high level of metabolic processes. They use about 10 % of all $O_2$, which used in organism. During 24 hours through kidney pass 700-900 L of blood. The main fuel for kidney are carbohydrates. Glycolysis, ketolysis, aerobic oxidation and phophorillation are very intensive in kidney. A lot of ATP formed in result.

Metabolism of proteins also present in kidney in high level. Especially, glutamine deaminase is very active and a lot of free ammonia formed. In kidney take place the first reaction of creatin synthesis.

Kidney have plenty of different enzymes: LDG (1, 2, 3, 5), AsAT, AlAT. Specific for kidney is alanine amino peptidase, 3rd isoform.

Utilization of glucose in cortex and medulla is differ one from another. Dominative type of glycolysis in cortex is aerobic way and $CO_2$ formed in result. In medulla dominative type is anaerobic and glucose converted to lactate.

Two sources contribute to the renal ammonia: blood ammonia (is about one-third of excreted ammonia), and ammonia formed in the kidney. The predominant source for ammonia production within the kidney is glutamine, the most abundant amino acid in plasma, but a small amount may originate from the metabolism of other amino acids such as asparagine, alanine, and histidine. Ammonia is secreted into the tubular lumen throughout the entire length of the nephron. Secretion occurs both during normal acid-base balance and in chronic acidosis.Metabolic acidosis is accompanied by an adaptive increase in renal ammonia production with a corresponding increase in urinary ammonium excretion.

Kidney cortex like liver appear to be unique in that it possess the enzymatic potential for both glucose synthesis from noncarbohydrate precursors (gluconeogenesis) and glucose degradation via the glycolytic pathway. Gluconeogenesis is important when the dietary supply of glucose does not satisfy the metabolic demands. Under these conditions, glucose is required by the central nervous system, the red blood cells, and possibly other tissues which cannot obtain all their energy requirements from fatty acids or ketone body oxidation. Also, gluconeogenesis may be important in the removal of excessive quantities of glucose precursors from the blood (lactate acid after severe exercise for example). Although the biosynthetic pathways are similar, there are several important differences in the factors which regulate gluconeogenesis in the two organs. 1) The liver utilizes predominately pyruvate, lactate and alanine. The kidney cortex utilizes pyruvate, lactate, citrate, α-ketoglutarate, glycine and glutamine. 2) Hydrogen ion activity has little effect upon hepatic gluconeogenesis, but it has marked effects upon renal gluconeogenesis. Thus, when intracellular fluid pH is reduced (metabolic acidosis, respiratory acidosis or potassium depletion), the rates of gluconeogenesis in slices of renal cortex are markedly increased. The ability of

the kidney to convert certain organic acids to glucose, a neutral substance, is an example of a nonexcretory mechanism in the kidney for pH regulation.

***Regulation of urine formation.***

Na-uretic hormone (produced in heart) decrease reabsorbtion of $Na^+$, and quantity of urine increased.

Aldosteron and some other hormones (vasopressin, renin, angiotensin II) increase Na-reabsor**btion and decrease quantity of urine.**

***Role of kidney in acid-base balance regulation.***

Kidney have some mechanisms for maintaining acid-base balance. $Na^+$ reabsorbtion and $H^+$ secretion play very important role.

1. Primary urine has a lot of $Na_2HPO_4$ (in dissociated form). When $Na^+$ reabsorbed, $H^+$ secreted into urine and $NaH_2PO_4$ formed.

2. Formation of hydrocarbonates. Inside renal cells carboanhydrase forms from $CO_2$ and $H_2O$ $H_2CO_3$, which dissociated to $H^+$ and $HCO3^-$. $H^+$ excreted from cell into urine (antiport with $Na^+$) and leaded with urine. $Na^+$ connect with $HCO_3^-$, $NaHCO_3$ formed and go to the blood, thereupon acidity decreased.

3. Formation of free ammonia. $NH_3$ used for formation of $NH_4^+$ ($H^+$ ion associted), and different acid metabolites excreted as ammonia salts.

***Role of kidney in water balance regulation.***

Excessive entrance of water leads to dilution of extracellular fluid. Decreasing of osmolality inhibits secretion of antidiuretic hormone. Walls of collective tubules stay non-penetrated to water and dilutive urine formed.

If volume of blood circulation increases, circulation in kidney increases also and hyperosmotic medium of kidney medulla removed. Some substances in these conditions return into blood. So, excess of water carried with urine and a lot of soluble substances are reabsorbed into blood. After water loading stopped, hyperosmolality in kidney medulla returns for previous stage during some days.

## *Physical and chemical characteristics of urine.*

Urine amount (diures) in healthy people is 1000-2000 ml per day. Day-time diures is in 3-4 times more than night-time.

Normal colour of urine is yellow (like hay or amber), what is due to presence of urochrom (derivate of urobilin or urobilinogen). Some another colour substances are uroerythrin (derivate of melanine), uroporphyrines, rybophlavine and other. Colour depends from urine concentration.

Urine is transparent. This characteristic depends from amount of different salts (oxalates, urates, phosphates), amount of present epitelium cells and leucocytes.

Density of urine depends from concentration of soluble substances. Borders of variation are from 1002 to 1035 g/l. Near 60-65 g of hard substances are excreted with urine per day.

In normal conditions urine has acid or weak acid reaction (pH=5,3-6,8). This depends from presence of $NaH_2PO_4$ and $KH_2PO_4$.

Fresh urine has a specific smell, which is due to presence of flying acids. But a lot of microorganisms, which are present in urine, split urea and free ammonia formed.

## *Organic compounds of urine.*

**Proteins.** Healthy people excretes 30 mg of proteins per day. As a rule these are low weight proteins.

**Urea.** This is main part of organic compounds in urine. Urea nitrogen is about 80-90 % of all urine nitrogen. 20-35 g of urea is excreted per day in normal conditions.

**Uric acid.** Approximately 0,6-1,0 g of uric acid is excreted per day in form of different salts (urates), mainly in form of sodium salt. Its amount depends from food.

**Creatinin and creatin.** Near 1-2 g of creatinin is excreted per day, what depended from weight of muscles. This is the constant for each person. Men excrete 18-32 mg of creatinin per 1 kg of body weight per day, women – 10-

25 mg. Creatinin is non-reabsorbable substance, so this test used for evaluating of renal filtration.

**Amino acids.** Per day healthy person excretes 2-3 g of amino acids (free amino acids and different low weight molecule peptides). Also products of amino acids metabolism can be found in the urine.

**Couple substances.** Hypuric acid (benzoyl glycine) is excreted in amount 0,6-1,5 g per day. This index increases after eating a lot of berries and fruits, and in case of protein's decay in the intestines.

**Indican (potassium salt of indoxylsulfuric acid).** Per day excrition of indican is about 10-25 g. Increasing of indican's level in urine is due to inrtensification of decay proteins in the intestines and chronic diseases, which are accompanied by intensive decopmosition of proteins (tuberculosis, for example).

**Organic acids.** Formic, acetic, butyric, β-oxybutyric, acetoacetic and some other organic acids are present in urine in a little amount.

**Vitamines.** Almost all vitamines can be excreted via kidney, especially, water-soluble. Approximately 20-30 mg of vit C, 0.1-0.3 mg of vit $B_1$, 0.5-0.8 mg of vit $B_2$ and some products of vitamine's metabolism. These data can be used for evaluating of supplying our organism by vitamines.

**Hormones.** Hormones and their derivates are always present in urine. Their amount depends from functional state of endocrinal glands and liver. There is a very wide used test – determination of 17-ketosteroids in urine. For healthy man this index is 15-25 g per day.

**Urobilin.** Present in a little amount, gives to urine yellow colour.

**Bilirubin.** In normal conditions present in so little amount that cannot be found by routine methods of investigations.

**Glucose.** In normal conditions present in so little amount that cannot be found by routine methods of investigations.

**Galactose.** Present in the newborn's urine, when digestion of milk or transformation of glalactose into glucose in the liver are violated.

**Fructose.** It is present in urine very seldom, after eating a lot of fruits, berries and honey. In all other cases it indicates about liver's disorders, diabetes mellitus.

**Pentoses.** Pentoses are excreted after eating a lot of fruits, fruit juices, in case of diabetes mellitus and steroid diabetes, some intoxications.

**Ketone bodies.** In normal conditions urine contains 20-50 mg of ketone bodies and this amount cannot be found by routine methods of clinical investigations.

**Porphyrines.** Urine of healthy people contains a few I type porphyrines (up to 300 mkg per day).

***Inorganic compounds of urine.***

Urine of healthy people contains 15-25 g of inorganic compounds.

**NaCl.** Per day near 8-16 g of NaCl excreted with urine. This amount depends from amount of NaCl in food.

**Potassium.** Twenty-four hours urine contains 2-5 g of K, which depends of amount of plants in the food.

Different drugs can change excretion of Na and K. For example, salicylates and cortikosteroids keep Na and amplify excretion of K.

**Calcium.** Twenty-four hours urine contains 0.1-0.3 g, which depends from content of calcium in the blood.

**Magnesium.** Content of magnesium in urine is 0.03-0.18 g. So little amount of calcium and magnesium in urine can be explained by bad water solubility of their salts.

**Iron.** Amount of iron in urine is about 1 mg per day.

**Phosphorus.** In urine are present one-substituted phosphates of potassium and sodium. Their amount depends from blood pH. In case of acidosis two-substituted phosphates ($Na_2HPO_4$) react with $H^+$ and one-substituted phosphates ($NaH_2PO_4$) formed. In case of alkalosis one-substituted phosphates react with bases and two-substituted phosphates formed. So, in both cases amount of phosphates in urine increases.

**Sulfur.** Amount of sulfur in twenty-four hours urine is 2-3 g per day (in form of $SO_4^{2-}$).

**Ammonia.** Ammonia is excreted in ammonium sulfates and couple substances. Ammonium salts make up 3-6 % of all nitrogen in urine. Their amount depends from character of food and blood pH.

## Introduction

The concept of clearance is central to three major areas of nephrology. First, the nature of urine for mation was explored to a great extent using clearance techniques. Second, the early search for measures of kidney function with advancing disease resorted to clearance procedures, particularly involving urea and creatinine. Third, the physiology of the kidney was examined and developed with great power and sophistication by the deepening theoretical understanding of the concept of clearance accompanied by ingenious analytical techniques and procedures. There is an additional domain which hovered over the studies of urine formation. This had to do with the per vasive resort to vitalism as an explanation of physiologic regulation. My task was to examine the birth and evolution of the clearance concept. For purposes of exposition, it may be helpful to describe first the clearance concept as it is currently understood and then recount how the concept emerged and developed during the 19th and early 20th centuries, as it was repeatedly invoked to analyze the process of urine formation, the failure of renal function, and the nature of physiologic regulation.

## THE CONCEPT OF CLEARANCE

Figure 1 gives the definition of clearance in currently conventional units of time and concentration. It is evident from the formula that the numerator is a rate of excretion (mg/mm); the denominator is a plasma concentration (mg/mL). Therefore, the clearance of any substance is expressed as mL/min (mg/min x

mL/mg=mL/min). Clearance, therefore, has the dimensions of a volume per unit time. This simultaneous measurement of the excretion rate of a solute and a flow rate of fluid from which the solute is derived has resulted in some confusion. Fig 2, modified from Cassin and Vogh, emphasizes that the kidney removes (clears) a small fraction of a substance from each mL of total flow. The clearance, therefore, of any substance is the virtual volume of plasma flow required to supply the amount of the substance excreted in any one minute.

## NATURE OF URINE FORMATION

The historical evolution of the clearance concept is intimately connected with studies examining the nature of urine formation. Particularly noteworthy reviews have been published by Smith (2), Bradley (3), Thurau, Davis and Haberle (4), Gottschalk (5), Schuster and Seldin (14). In the early 19th centur y, Johannes Muller (18011858) advanced a theor y of urine formation that rested on two prevalent concepts current at the time: 1) fluid movement was a secretory process mediated by glands; 2) the activity of the secretor y system required vitalistic forces that could not be reduced to physical processes. Despite enormous contributions to microscopic anatomy, he denied that the glomerulus was directly connected with the renal tubules, and ascribed urine formation to the secretory activity of the tubules, regarding the kidney as a gland. Notwithstanding the powerful currents of vitalism at the time, Carl Ludwig (1816-1895) came to the study of kidney function with an uncompromising physicochemical orientation. He appreciated the role of the afferent and efferent arteriole in elevating the hydrostatic pressure in the intervening glomerulus, thereby facilitating the movement of a protein-free ultrafiltrate, containing all the elements to be found in the urine, and restraining the passage of protein and formed elements. To account for the different composition of blood and urine, Ludwig proposed that some unspecified chemical force promoted active sodium chloride reabsorption while some property of the tubular wall restrained urea back-diffusion. No vital force

was postulated, although the nature of the "chemical force" promoting reabsorption was unspecified. Simultaneously and independently, William Bowman (1816-1892) also postulated that water was separated from blood at the glomerulus, but he assumed that solutes remained in the blood and were subsequently secreted into the urine by the tubules. This was an expression of the prevalent view of glandular secretion mediating solute movement. The central feature of Ludwig's theory that urine formation was critically linked with glomerular pressure, was challenged by Rudolph Heidenhain (1834-1897). On the basis of calculations of a clearance type, he concluded that to attribute urea excretion to filtration alone would require 70 liters of filtrate and 68 liters of water reabsorption per day. Heidenhain concluded that such circumstances were inconceivable, that urine formation was linked with renal blood flow, not filtration pressure, and that urine was derived from secretion by both glomerulus and tubules. When Cushny (1866-1926) introduced his "modern view" in his monograph of 1917, he accepted Ludwig's thesis that the ultimate source of urine was filtration at the glomerulus of non-colloid constituents by a purely physical process. He rejected Heidenhain's calculation as involving far too low an estimate of renal blood flow; in addition, when the immense number of renal tubules are taken into account, the requirement for water reabsorption could be reasonably accounted for. Cushny rejected entirely the notion of tubular secretion. To account for reabsorption, Cushny postu1ated some "vital activity" on the part of the epilthelium – a postulate that Ludwig had steadfastly rejected. Thurau and his associates (4) have pointed out that these various theories of urine formation were unifed in a perceptive analysis by Metzner (1858-1935) published in 1906, some ten years before Cushny's "modern theory".

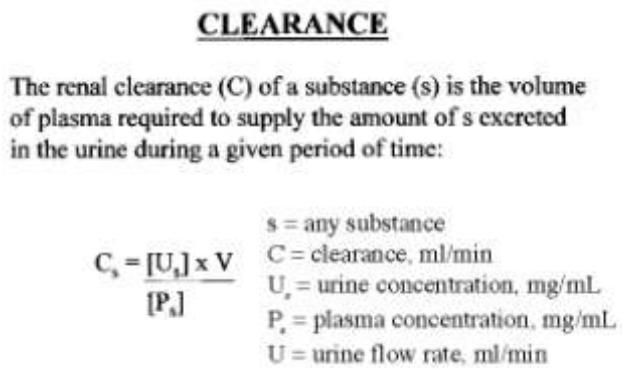



**Fig. 1**

His conception of a trifold process of ultrafiltration at the glomerulus by physical forces, reabsorption of most of the filtrate in part by active tubular processes, and active secretion of certain solutes by the tubular epithelia is remarkably close to modern views. His summary is worth quoting (Fig. 3). It should be emphasized that the use of clearance calculations by Heidenhain and their reinterpretation by Cushny served to make creditable the conceptual model of the comparatively modest magnitude of urine flow in a setting of huge volumes of glomerular filtration.

## DIGRESSION ON VITALISM

Although Cushny may have over-emphasized the commitment of Heidenhain and others to vitalism, there is no question that the concept of vital activity, not reducible to physical forces, was a powerful conceptual factor that infected the theories of renal function. For most of the 19th centur y, a basic problem in biology was conceived to be the distinction between living and non-living matter. A mechanistic explanation assumed that organic and non-organic matter were not irreducibly different. A vitalistic explanation assumed that a reduction of living to non-living phenomena is in principle impossible. Embryology was a dominating biologic discipline. To provide a flavor of the intellectual climate surrounding the study of renal function, it may be helpful to review briefly the prevailing embryologic studies. Landmark studies exemplified by the work of Hans Driesch

are summarized in a comprehensive publication in 1914 (7). In a series of studies on embr yonic sea-urchins, he demonstrated that rearrangement of cells at the blastomere stage had no effect on normal development. Moreover, a single blastomere, isolated from the rest at the two - or fourcell stage, can develop into a normal sea-urchin embr yo.

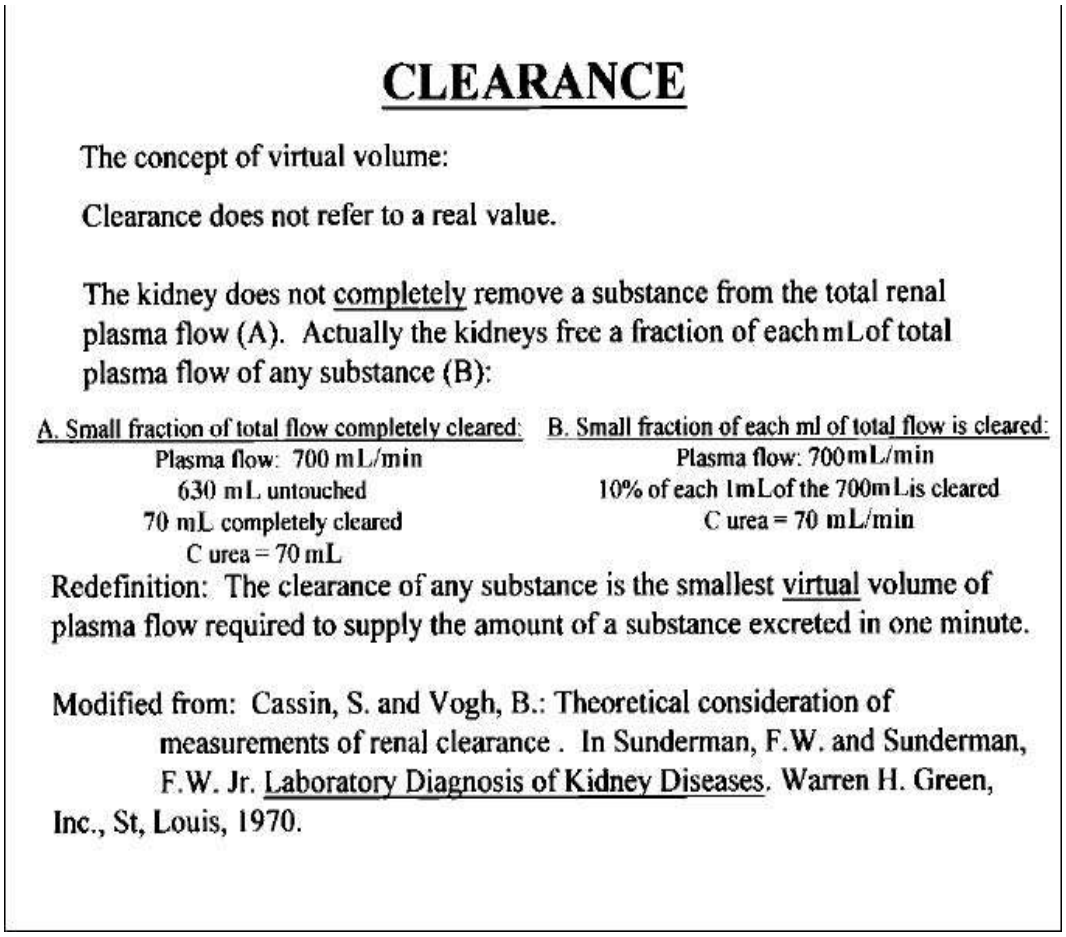



**Fig. 2**

The conclusion was drawn that spatio temporal location is irrelevant to development, and that non-physical forces “entelechies” are “wholemaking” factors which have no quantitative characteristics. It was only the gradual advancement of physical and biologic science that could meet the vitalistic

arguments. Organic chemistr y was shown to be a misnomer. The synthesis of urea, heretofore found only in living organisms, from $CO_2$ and NHby3 Wöhler in 1828 (7) led to the view that organic chemistr y was simply the chemistr y of carbon compounds. Purpose and purposiveness were explained by reference to integrated and adjustible feed-back systems. The ability of blastomere cells to develop differently in different transplant locations in ontogenesis, unlike a machine where each part fulfills a designed function, is explainable in principle by genetic theory. And finally "energy" input required to impart selectivity is not confined to hydrostatic or oncotic forces. On a conceptual level, it was pointed out by the logical positivist philosopher C.I. Hengel that vitalism has no predictive power, offering neither verifiable predictions nor providing models of coherent mechanisms. It was the increasing power of the physical sciences that gradually undermined the recourse to postulated entities which could not be identified, characterized, or worst of all, refuted. It was these reasons which led Ludwig and Cushny to vigorously reject vitalistic explanations.

## UREA AND UREA CLEARANCE

Bright in 1836 recognized that the concentration of blood urea rose in patients with chronic renal disease (3). Ambard (8) showed that the blood level of urea was related to urea excretion and formulated an equation which was designed to register impairment of renal function.

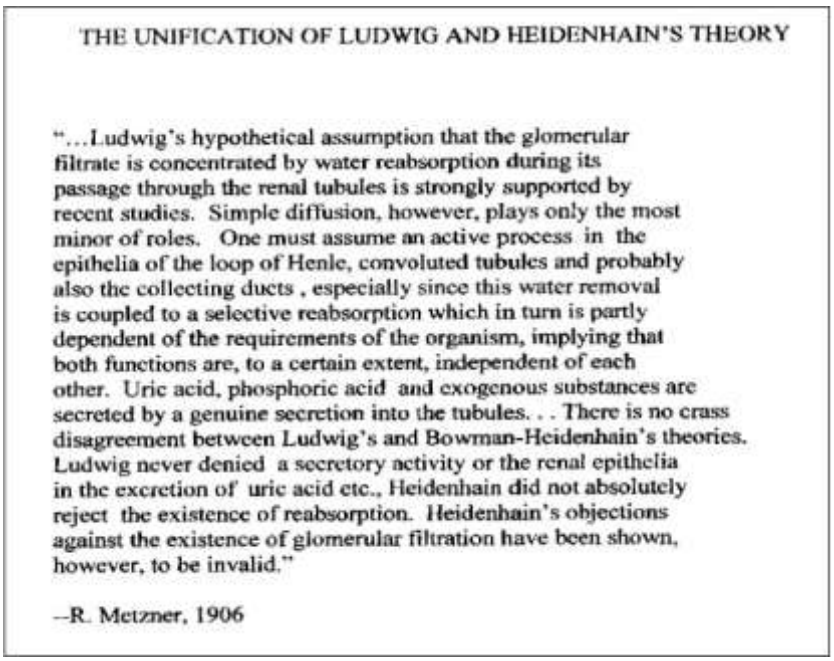



**Fig. 3**

However, the equation involved a square root function which obscured the physiologic significance of the relationship between urinar y excretion and blood urea concentration. Addis (9), in 1917, showed that at maximal urine flows the ratio of the excretion of urea per hour and the blood urea concentration was constant in any one individual. This expression represented the urea clearance per hour, an approximation of glomerular filtration rate. Austin, Stillman and Van Slyke (10) showed that the rate of urine flow influenced urea excretion independently of the blood level and renal excretory capacity. In a later study (11), it was demonstrated that above urine flows of 2ml/min (augmentation limit), the relationship between urea excretion and plasma concentration in any one individual was constant, and expressed by a simple formu1a:

$$C_{urea} = U_{urea} / P_{urea}$$

The term, clearance, was introduced with this analysis. Since blood urea concentration is frequently used as an index of filtration rate, it is worthwhile examining the factors which influence it independently of intrinsic renal function. It has alredy been pointed out that urine flow influences urea clearance. Urea undergoes a complex intrarenal recycling process, the fractional reabsorption increasing from 35% of the filtered load in hydrated states to 60% in dehydration. The blood urea concentration is influenced by a variety of factors independent of renal function. Changes in urine flow affect blood urea in a manner which depends on the nephron segment where fluid is being reabsorbed.

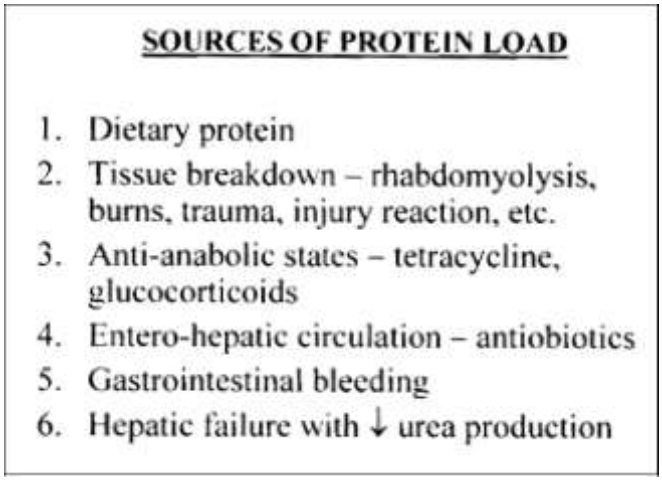



**Fig. 4**

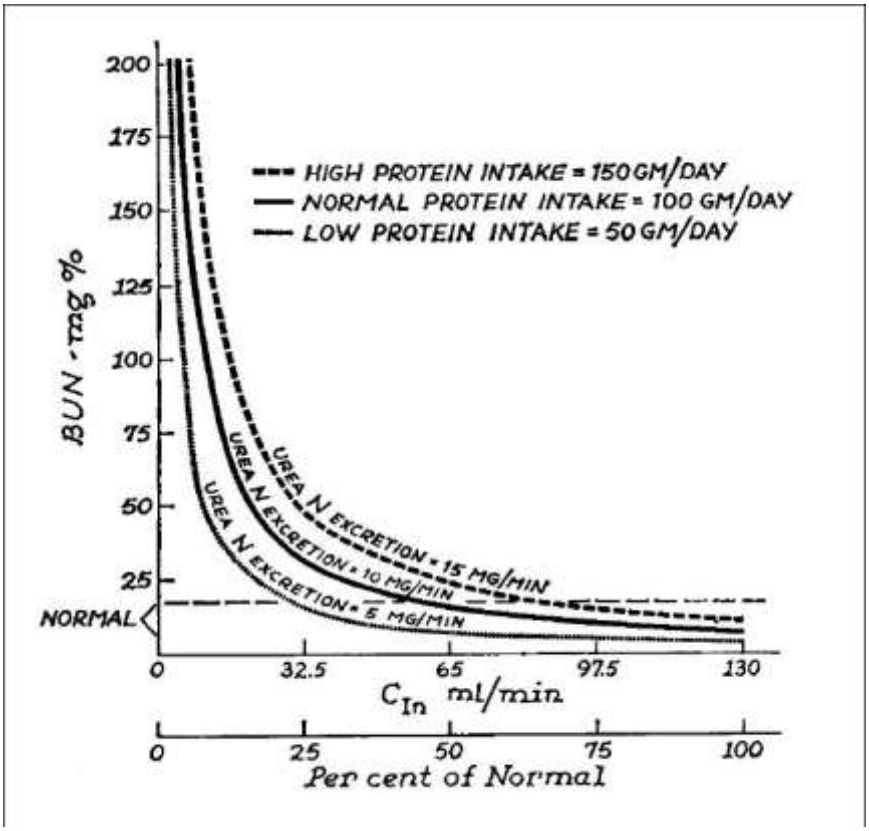



**Fig. 5**

The proximal tubule is highly permeable to urea, and is the principal segment of passive reabsorption. The distal nephron is less permeable to urea, even in the presence of antidiuretic hormone. If ever ything else is left constant, salt depletion will produce more azotemia at the same low rate of urine flow than will water restriction, because salt depletion accelerates proximal reabsorption while water restriction accelerates principally distal reabsorption (12). Protein loads also influence blood urea concentration independent of renal function. Figure 4 lists the sources of protein loads. Factors 1-5 serve to increase protein loads while factor 6 reduces it. Figure 5 (13) illustrates the effect of protein intake at various levels of renal function.

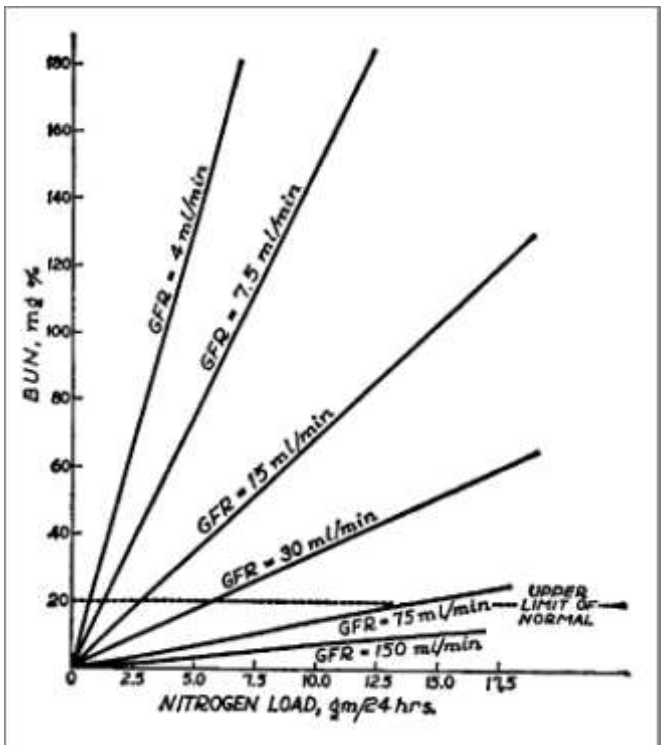



**Fig. 6**

It should be emphasized that at low filtration rates, the BUN is very sensitive to protein loads, as is illustrated by Figure 6 (13). Figure 7 summarizes the various factors which influence both the BUN and urea clearance. It is evident that the interpretation of the BUN as a rough index of GFR requires correction for the numerous factors which influence its concentration independent of renal function. Urea clearance circumvents some of the distorting effects of protein loads. Nevertheless, it is always less than inulin clearance, but tends to rise toward inulin clearance with advanced renal failure. These various factors are discussed in detail in ref. 14.

## PHYSIOLOGY OF THE KIDNEY

Although Addis and Van Slyke had published landmark studies on urea clearance as a measure of renal function, the precise relation between urea clearance and glomerular filtration rate was not appreciated. Rehberg (15, 16) introduced creatinine as a marker of glomerular filtration rate, but was unaware that it was

secreted by the tubules, and therefore would give falsely high values, especially if its plasma concentration was raised by infusions. Smith was skeptical that creatinine would be an ideal marker for glomerular filtration, since it underwent secretion in aglomerular fish, and might do the same in mammals, a supposition that proved correct. He then went on to elaborate the criteria for an ideal marker of GFR (Fig. 8).

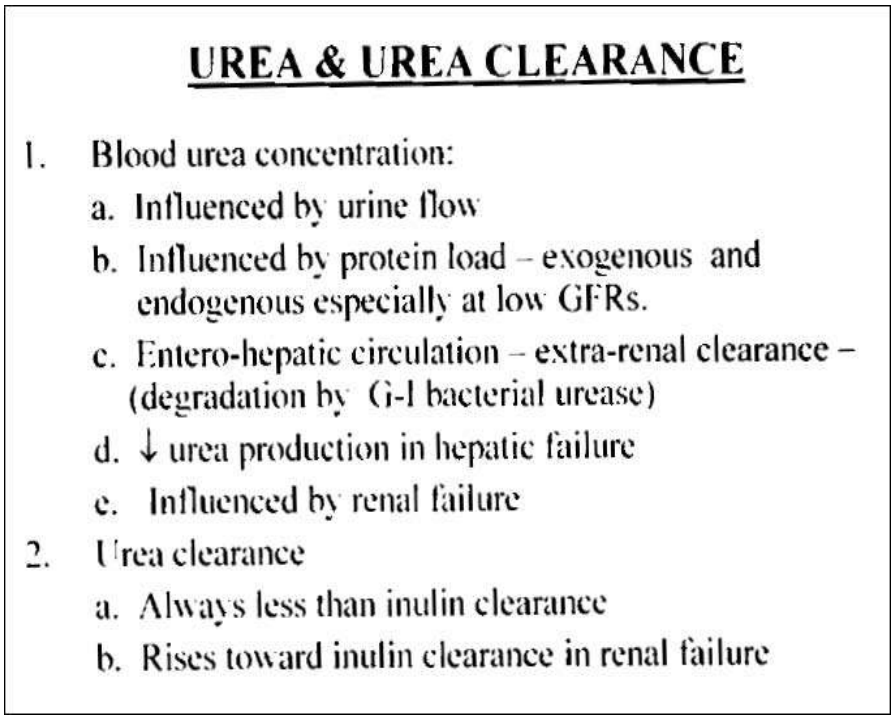



**Fig. 7**

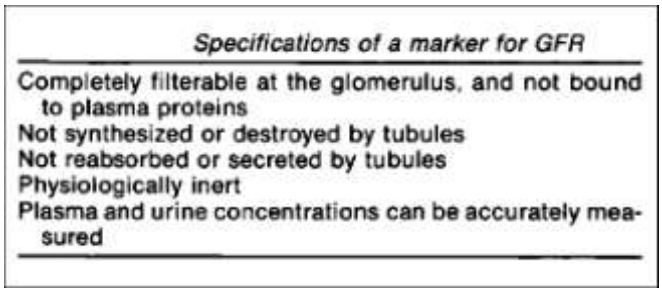
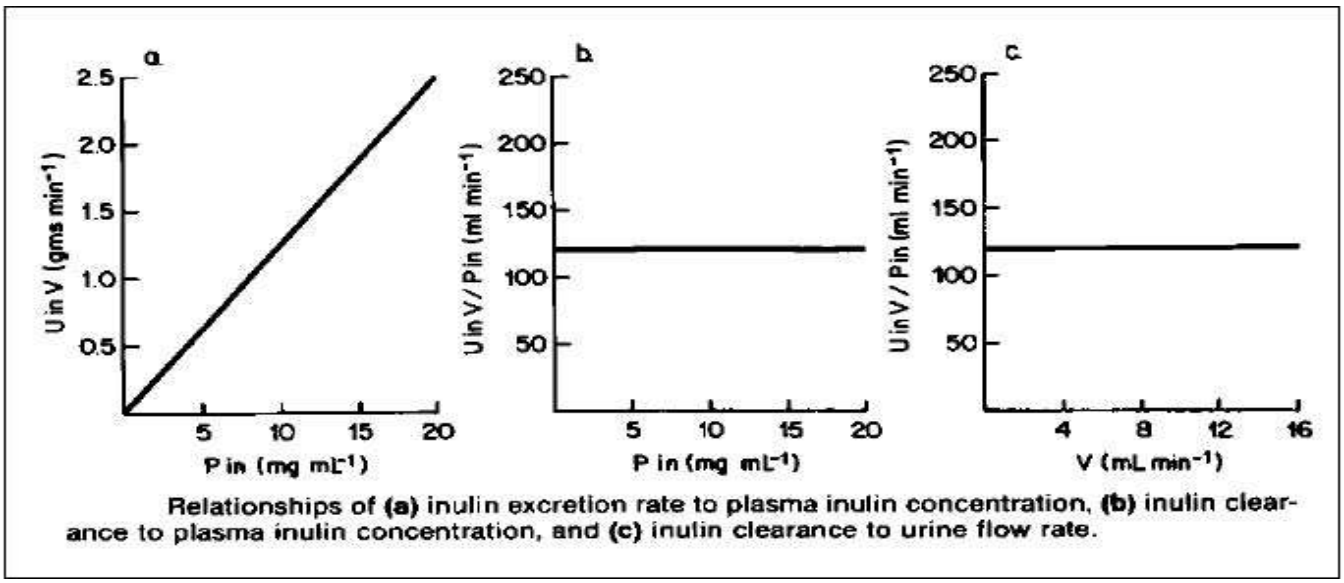



**Fig. 8**

The failure of sugars to be secreted in aglomerular fish led Smith ultimately to identify inulin as an ideal marker (17, 18). Simultaneously and independently, Richards and his associates also demonstrated in micropuncture studies that inulin fulfilled the requirements for an ideal marker of GFR (19). In Figure 9, inulin excretion is shown to increase in direct proportion to its plasma concentration when GFR is constant (a); inulin clearance is constant over a wide range of plasma inulin concentrations (b); inulin clearance is constant over a wide range of urine flows (c). Findings such as these established inulin as a kind of gold standard for GFR (14). Smith went on to develop methods for measuring renal blood flow, utilizing diodrast as a marker and the Fick principle to calculate total renal blood flow. Since the Fick principle required renal vein catheterization, it was unsuitable for routine use. Para-amino hippurate (PAH) was identified as a substance which, at low plasma concentrations, was almost completely secreted into the tubular urine. This eliminated the need for renal catherization, since renal venous PAH could be assumed to be close to zero. Subsequent studies of tubular maximum transport capacity using glucose and many other substances provided a measure of functioning tubular mass.



Relationships of **(a)** inulin excretion rate to plasma inulin concentration, **(b)** inulin clearance to plasma inulin concentration, and **(c)** inulin clearance to urine flow rate.

**Fig. 9**

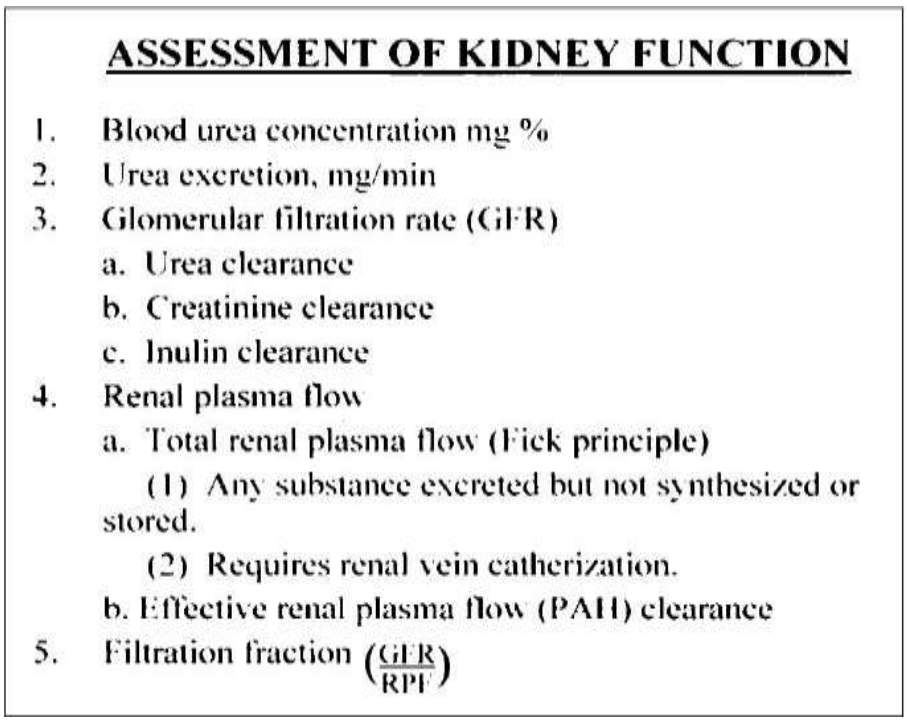



**Fig. 10**

These various measures allowed Smith to portray the various functional aspects of normal and diseased kidneys in remarkable detail, as summarized in his Porter lectures (20). From a clinical and physiologic point of view, the various measures of renal function that Smith and others explored over the years are summarized in Figure 10. Smith has remarked how fruitful the clearance concept has proved to be. From rather tentative beginnings it has stimulated the search for novel analytic procedures, allowed for the assessment of renal function, and most of all provided a conceptual rallying point for insight and understanding of renal physiology.

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