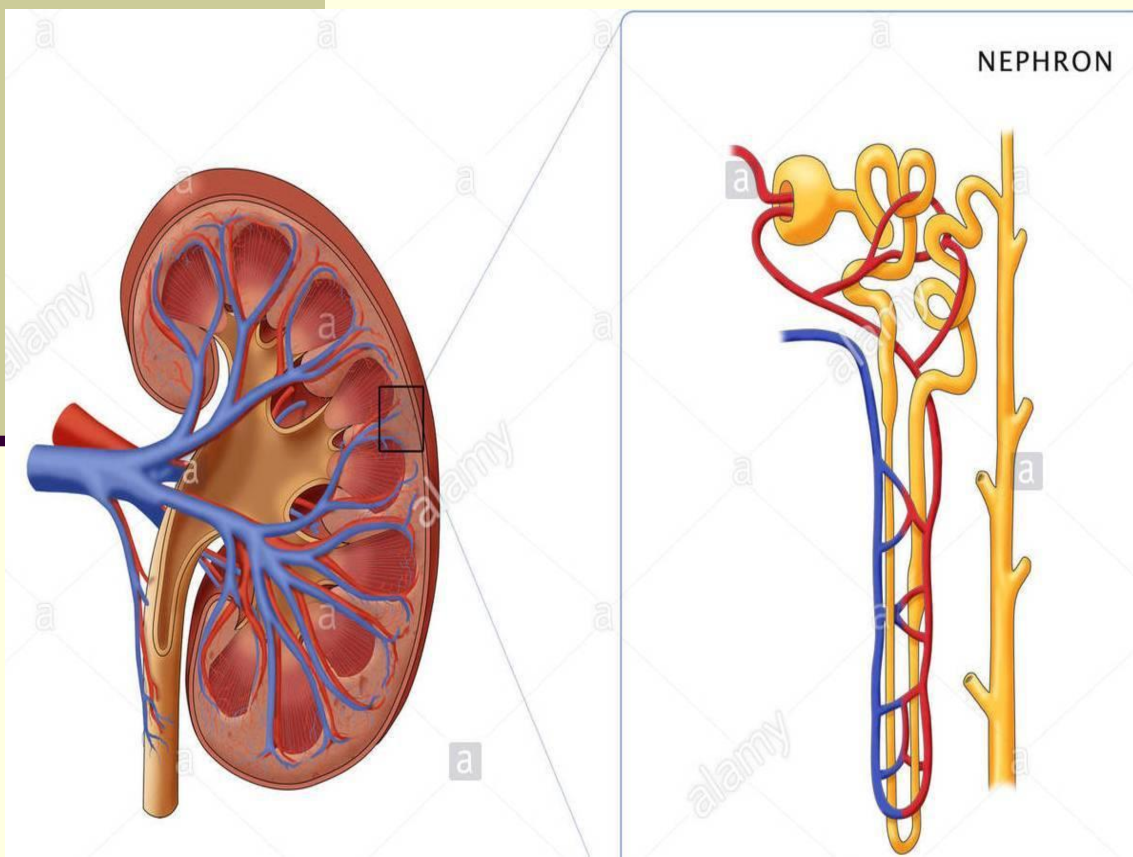
BIOCHEMISTRY OF THE KIDNEY AND URINE



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I.

Kidney, biochemical functions, metabolism of the kidney. Role of kidney in regulation of pH balance

Morphology of the urinary system

- Organ system that produces, stores, and carries urine
- Includes two kidneys, two ureters, the urinary bladder, two sphincter muscles, and the urethra.
- Humans produce about 1.5 litres of urine over 24 hours, although this amount may vary according to the circumstances.
- Increased fluid intake generally increases urine production.
- Increased perspiration and respiration may decrease the amount of fluid excreted through the kidneys.

Kidneys

- Is the paired organ (weight about 300 g)
- Its parenchyma is divided into two major structures:
  - the outer - renal cortex
  - the inner - renal medulla.
- Consists of about 1 million filtering units termed nephrons (basic structural and functional unit)

RENAL FUNCTIONS

1. **Formation of urine and excretion the waste metabolic products from the body** ***(ammonium, urea, uric acid, creatinine, etc.).***

2. **Reabsorption and preservation of vital nutrients** ***(glucose, proteins, amino acids, etc).***

3. **Regulation of water and electrolyte balance** ***(Na+, K+, Cl-)***

RENAL FUNCTIONS *(Cont.-d)*

4. **Maintenance of pH homeostasis in the blood plasma** ***(via renal mechanisms).***

5. **Regulation of blood pressure** ***(via maintaining the electrolyte and water balance, role of renin).***

RENAL FUNCTIONS (*Cont.-d*)

6. **Metabolic function:** ***synthesis of specific proteins and other compounds; catabolism of lipids, carbohydrates, energy metabolism.***

7. **Endocrine function:** ***synthesis of hormone-like substances***
   - *renin*
   - *Erythropoietin*
   - *1-α,25-dihydroxycholecalcipherol*

• *Renin*

- is the enzyme that is synthesized by the juxtaglomerular cells in the kidneys from prorenin, already present in the blood.
- It is the part of the RENIN–ANGIOTENSIN SYSTEM (RAS) and that regulates blood pressure and fluid balance.
- renin is then secreted into the circulation and catalyzes conversion of angiotensinogen into angiotensin-I.

• *Erythropoietin*

- **Polypeptide hormon that is formed predominantly by the kidney (also by the liver)**
- **It controls the differentiation of the bone marrow stem cells.**
- **The release is stimulated by hypoxia (low pO_2).**
- **The hormon ensures that the bone marrow cells are converted to erythrocytes, so that their concentration in the blood increases (erythropoiesis)**

• *1 alpha,25-dihydroxycholecalcipherol*

- steroid-related hormon involved in calcium homeostasis.
- active form of VITAMIN D_3 (CALCITRIOL)
- increases the level of Ca^{2+} in the blood
- increases the uptake of Ca^{2+} from the intestine into the blood
- increases reabsorption of Ca^{2+} by the kidneys

2. Metabolism in kidneys

The physiological processes that occur in the kidney require a large supply of energy.

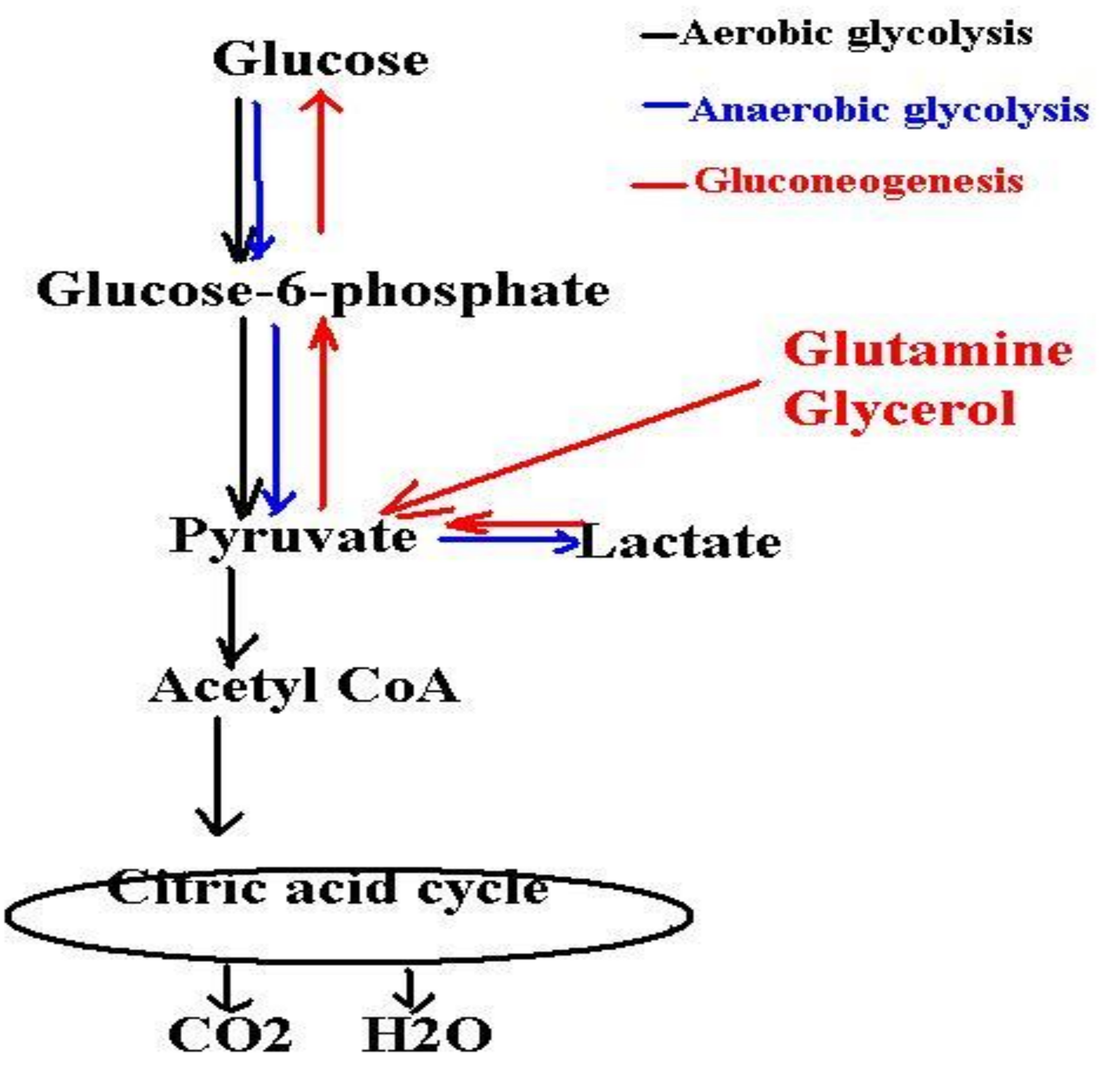
- High intensity of energy metabolism

THE MOST ACTIVE METABOLIC PATHWAYS

- aerobic glycolysis, gluconeogenesis
- β-oxidation of fatty acids
- Utilization of ketone bodies
- Transamination and deamination of amino acids.
- Energy dependent transport of ions, nutrients, waste products, etc (filtration and reabsorption).

Fate of glucose in the kidneys

No pentose-phosphate pathway and synthesis of glycogen



Carbohydrate metabolism in the kidney

- **Renal cortex - more aerobic glycolysis,**
- **Renal medulla – more anaerobic glycolysis**

Gluconeogenesis

- **Take place in renal cortex only**
- **The main substrate is glutamine**
- **Other substrates are amino acids, lactate, glycerol or fructose (all are obtained from the blood plasma)**
- **The ammonia derived from this glutamine serves to buffer the pH of the urine.**
- **Gluconeogenesis is regulated by insulin, adrenalin, and induced by cortisol.**

Energy metabolism

- Most metabolic processes in the kidney are aerobic, and oxygen consumption in the renal tissue is very high.
- The kidney consumes about 10% of all O_2 in the body;
- About 70% of all that O_2 is used for generation of energy for maintaining of tubular reabsorption of glucose and amino acids.

Energy sources of the kidney

- About 90% of ATP is derived from β-oxidation of fatty acids and utilization of ketone bodies.
- The rest 10% of ATP is derived from oxidation of glucose, lactate, pyruvate, glycerol, citrate and amino acids absorbed from the blood.
- Most of all ATP available in the renal tubular cells is used for functioning of Na^+/K^+-ATPase responsible for reabsorption of glucose and amino acids.

Lipid metabolism in the kidney

- From all lipids
  - 80-85% are phospholipids and cholesterol
  - 10-15% are TAGs
- Higher rate of β- oxidation of fatty acids and utilization of ketone bodies
- Place of utilization of plasma lipoproteins.

Metabolism of amino acids in the kidney

- Regulation of plasma concentrations of amino acids
- About 70 g of amino acids per day, derived from the diet and metabolism in the liver, muscle and other tissues, are filtered from the arterial blood by the kidney. Most of these amino acids are actively reabsorbed in the proximal tubules and after metabolism leave the kidney by the renal vein.
- The most metabolized amino acids are Glutamine, Glutamate, Aspartate, Glycine.
- Glutamine is used for regulation of pH of blood plasma.

Metabolism of amino acids in the kidney (cont.-ed)

- **High activity of transaminases and glutamate dehydrogenase (transamination and deamination of amino acids).**
- **Ammonia produced in the kidneys is used for regulation of the acid-base balance.**
- **First reaction in the synthesis of creatinine.**

Arginine + glycine → guanidine acetate

Metabolism of proteins in the kidney

- The kidney is a major site for the catabolism of both circulating and kidney peptides and proteins
- Of circulating proteins the kidney breaks down small and medium-sized blood plasma proteins (below 6000 Da), such as insulin and peptide hormones.

MECHANISMS FOR REGULATION OF pH

- The acid-base balance in the blood and extracellular fluids is maintained by cooperative interaction of three main mechanisms:
  - buffer systems of the blood;
  - respiratory mechanisms,
  - renal mechanisms.
- The effect of buffer systems of the blood is manifested within 30 seconds.
- The lungs require a period of 1-3 min to normalize pH in the blood.
- Kidney needs 10-20 h to restore a disturbed acid-base balance.

Renal mechanisms of acid-base homeostasis (pH balance)

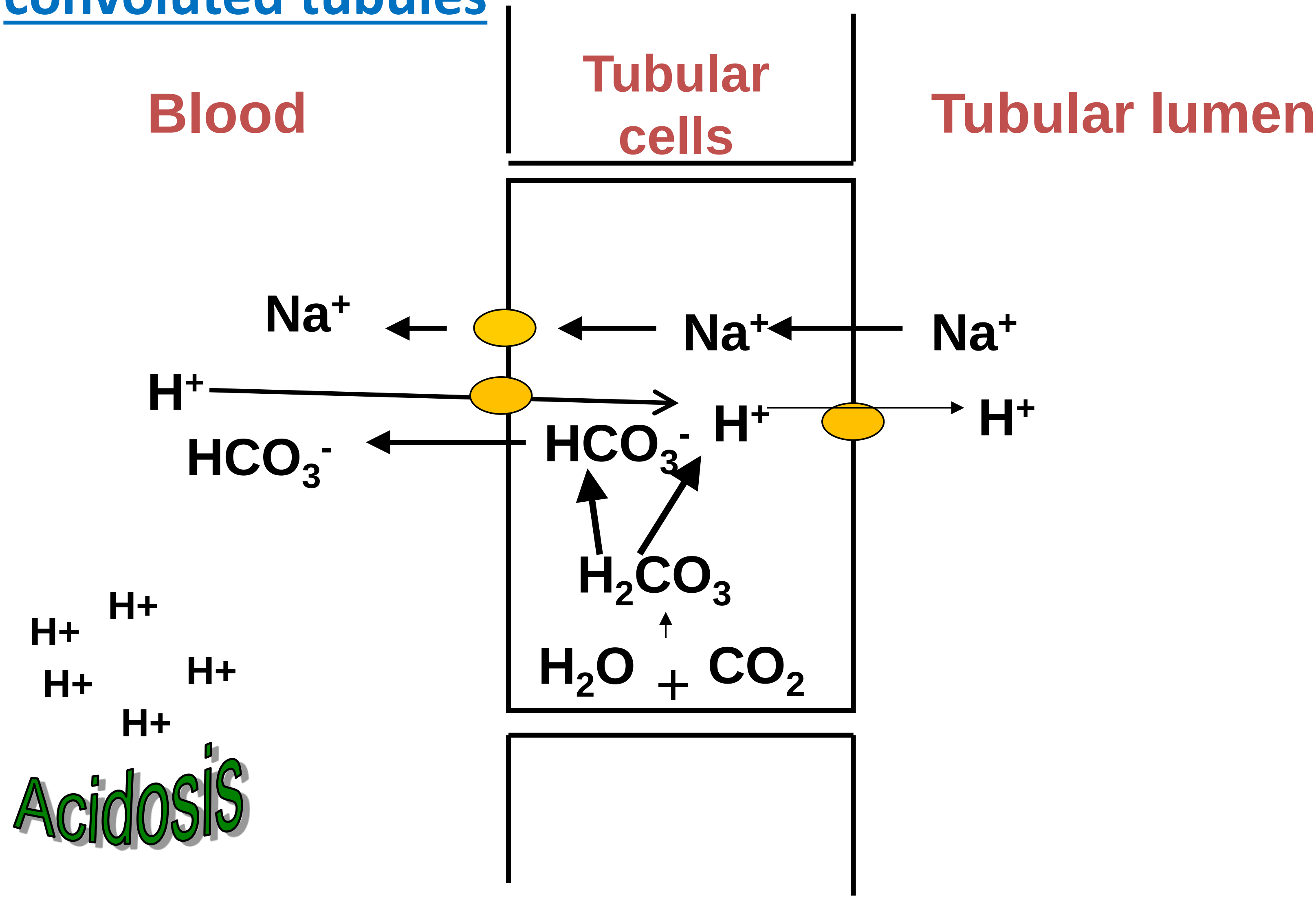
The kidney regulates pH of the blood plasma by:

- **Reabsorption of bicarbonate ions (HCO_3^-)**
- **Excretion of hydrogen ions (H^+)**

3 mechanisms provide excretion of excessive hydrogen ions from blood:

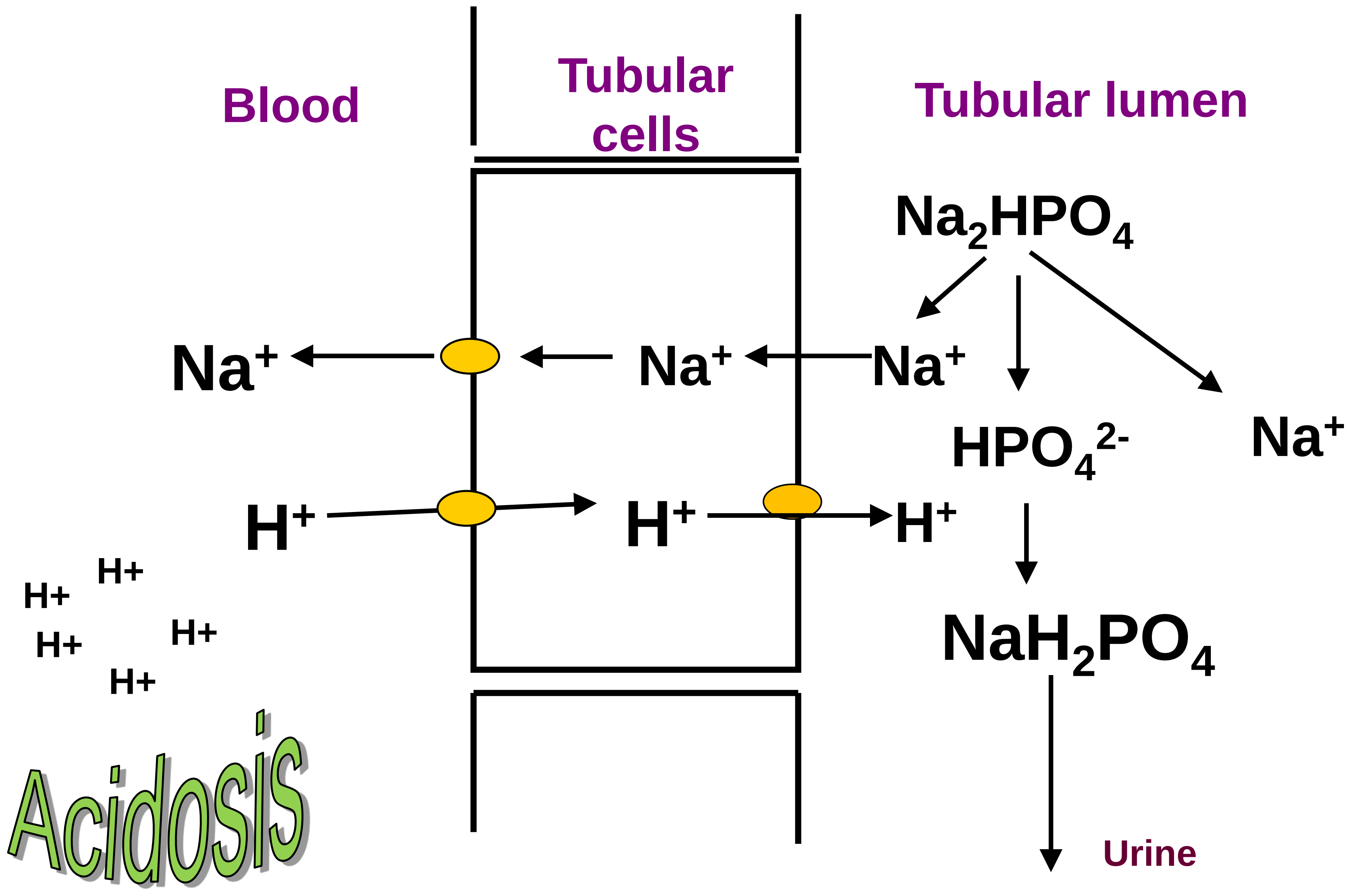
I. **Reabsorption of bicarbonate ions**
II. **Excretion of H^+ via phosphate buffer system ($H_2PO_4^-$/ HPO_4^{2-})**
III. **Excretion of H^+ as ammonium ion (NH_4^+)**

Mechanism 1. Formation of carbonic acid with the following dissociation to H^+ and HCO_3^- in the proximal convoluted tubules



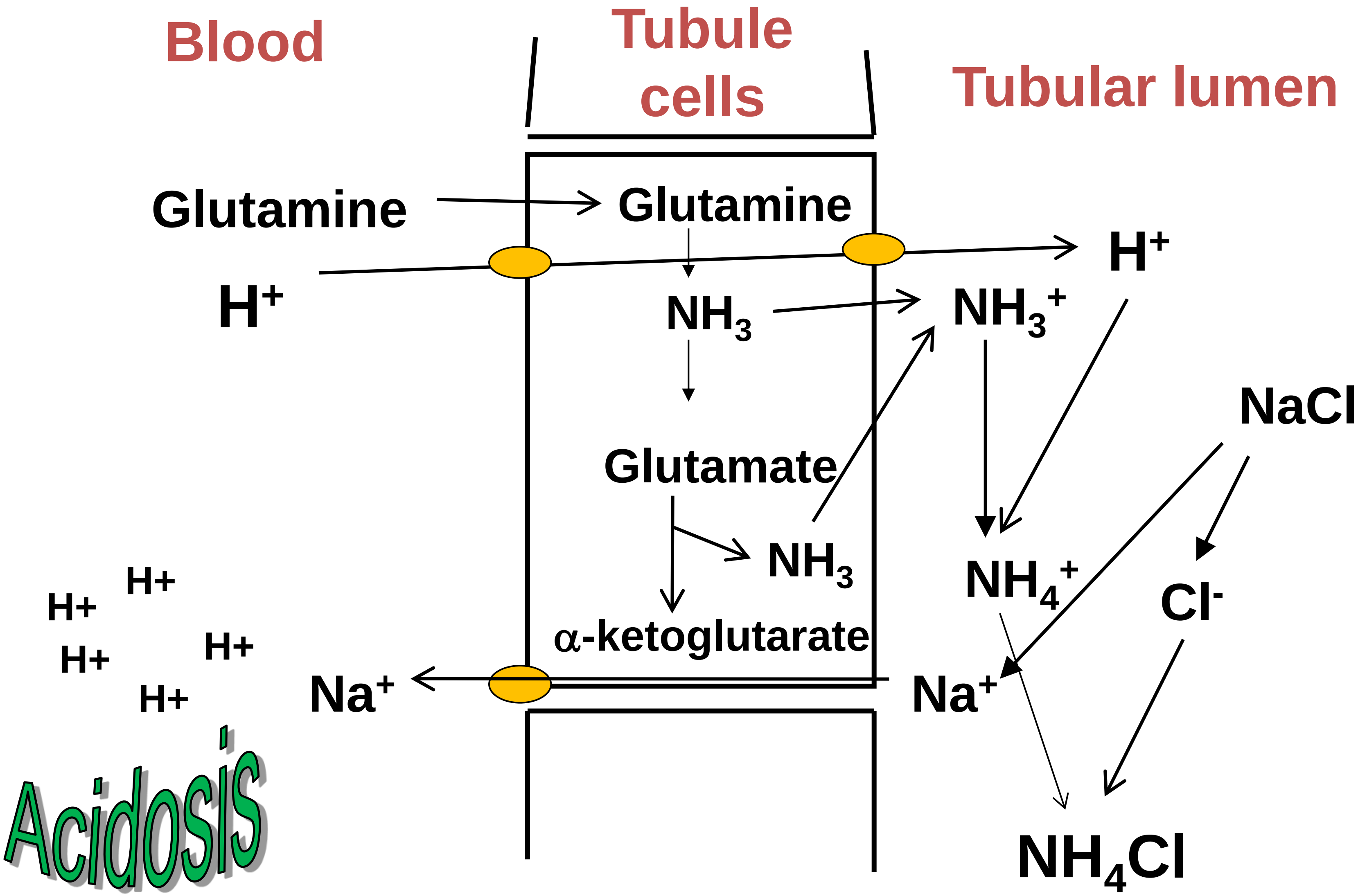
1. CO_2 and H_2O are produced in the mitochondria of tubular cells.
2. Next the enzyme carbonic anhydrase binds CO_2 to H_2O to form H_2CO_3
3. Carbonic acid easily dissociates to give HCO_3^- and H^+.
4. H^+ from the blood and tubule cells are secreted to the lumen in exchange for Na^+.
5. As the result of these events hydrogen ions (H^+) are eliminated from organism, and HCO_3^- is reabsorbed in plasma along with Na^+.
6. THIS MECHANISM SERVES TO INCREASE THE ALKALI RESERVE OF THE BLOOD PLASMA AND TO PREVENT THE LOSS OF BICARBONATE WITH THE URINE.

Mechanism 2. Hydrogen phosphate ion is converted into dihydrogen phosphate <u>in the distal convoluted tubules</u>



1. The renal filtrate produced in the glomeruli contains Na_2HPO_4.
2. In acidosis excessive amounts of H^+ are secreted from blood and the tubular cells in exchange for Na^+.
3. In tubular lumen dihydrogen phosphate (HPO_4^{-2}) reacts with Na^+ and H^+ ions to form NaH_2PO_4.
4. Sodium dihydrophosphate (NaH_2PO_4) is then excreted through the urine.
5. THIS MECHANISM ALLOWS TO REABSORB AND SAVE SODIUM IONS AND AVOIDS DEHYDRATION.

Mechanism 3. Excretion of hydrogen ions as ammonium salts in the distal tubules



1. In acidosis the uptake of glutamine and H^+ from blood to the tubule cells is increased.
2. Also acidosis stimulate activity of the kidney glutaminase and glutamate dehydrogenase, the enzymes that produce NH_3 from glutamine and glutamate.
3. Non-ionized ammonia can easily diffuse through cell membrane into the tubular lumen. In turn H^+ diffuse into the lumen in exchange for Na^+.
4. In the tubular lumen NH_3 binds with H^+ to form ammonium ion (NH_4^+), the latter is neutralized by Cl^-
5. Resulted AMMONIUM CHLORIDE is excreted through urine from the body.

II.

General characteristics and composition of urine. Pathologic components of urine. Role of urine analysis in diagnosis.

Urine formation

- For the production of urine, the kidneys do not simply pick waste products out of the bloodstream and send them along for final disposal.
- The kidneys' 2 million or more nephrons (about a million in each kidney) form urine by

3 precisely regulated processes

1. glomerular filtration
2. reabsorption
3. secretion

PHYSICAL CHARACTERISTICS OF THE URINE USED IN CLINICAL LABORATORIES

include **appearance**, **color, turbidity (transparency), smell** (odor), **pH** (acidity - alkalinity) and **volume.** Many of these characteristics are notable and identifiable by vision alone, but some require laboratory testing.

1. APPEARANCE (transparency):

Normal urine is clear and transparent

- **Cloudy → presence of oxalates, protein, cell elements, bacteria, etc.**

2. SPECIFIC GRAVITY (OR DENSITY) .

is the ratio of the weight of a volume of the urine compared with the weight of the same volume of distilled water.

Normal SG is most commonly 1.015-1.029.

↑ More concentrated urine, dehydration, excessive sweating, urinary tract infections.

↓ More diluted urine in renal failure, pyelonephritis, diabetes insipidus.

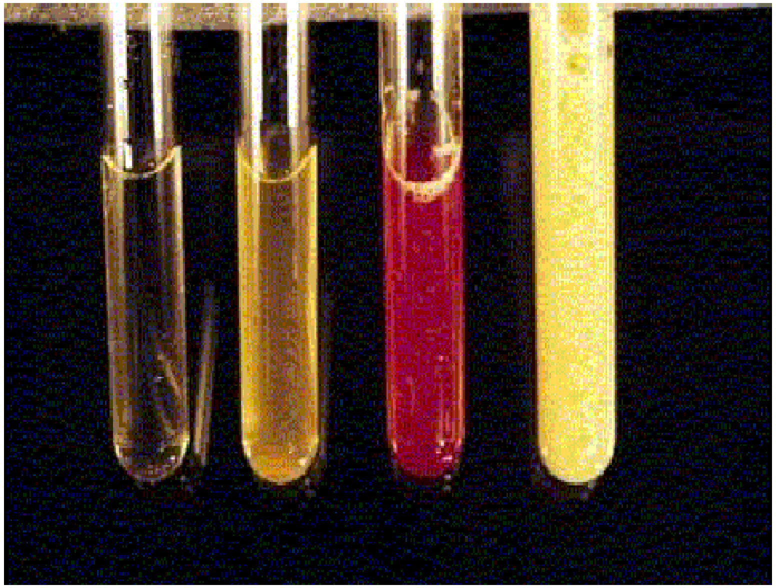
3. ODOUR:

- Smell of acetone: Diabetic ketoacidosis
- Fishy: Presence of blood

4.COLOUR:

Is Amber-yellow due to urochrome

- High colour → Jaundice
- Red colour → Blood
- Dark colour → dehydration
- Light colour → drinking more water



5. pH of the urine:

Ranges from 5.3 to 7 with an average of 6.2

- **high protein diets, alcohol → more acidic urine,**
- **vegetarian diets → more alkaline urine.**

Severe shifts in the pH values:

↓ Metabolic acidosis, diabetes mellitus, starvation, fever

↑ Metabolic alkalosis, cystitis, pyelitis.

6. VOLUME OF THE URINE

- **Normal - in woman - 1200 ml/day,**

  in men - 1500 ml/day.

- **Polyuria: ↑Urine output → > 2.5 litres/day**

Seen in: - increased water ingestion

- diabetes mellitus and insipidus

- **Oliguria: ↓ Urine output → 300 to 500 ml/day**

Seen in: - dehydration

- acute glomerulonephritis

- **Anuria: ↓↓↓ Urine output → 0 - 50 ml/day**

Seen in: - renal shutdown in acute renal failure

Daily diuresis

- **is increased urination and the physiologic process that produces such an increase. It involves extra urine production in the kidneys as part of the body's homeostatic maintenance of fluid balance**

Composition of the Urine

A sterile fluid composed of:

1. Water (about 95%)
2. Organic constituents
   (35-45 g/day)
3. Inorganic constituents
   (15-25 g/day)

Organic components of urine

Nitrogen-containing compounds

Urea (80%), uric acid, creatine, creatinine, AA, bilirubin, indican, etc.

Nitrogen free compounds

Organic acids

(Oxalic, lactic, citric, butyric, valeric, β-hydroxybutyric, acetoacetic acids)

Also pigments, hormones and their derivatives, etc.

Inorganic components of urine are the same as in the blood plasma

	mmol/day
Cl^-	120 – 240
Na^+	100 – 150
K^+	60 – 80
SO_4^{2-}	30 – 60
NH_4^+	30 – 60
HPO_4^{2-}	10 – 40
Ca^{2+}	4 – 11
Mg^{2+}	3 – 6

Pathological components of the urine

- are absent is the normal urine or present ONLY in trace amounts and increase in diseases.
  - Proteins (proteinuria)
  - Glucose (Glucosuria)
  - Ketone bodies (ketonuria)
  - Occult blood (hematuria)
  - Bile pigments.
  - Porphyrins

Causes of Proteinuria

- **There are three main mechanisms to cause proteinuria:**
  1. **Due to disease in the glomerulus (renal proteinurea)**
  2. **Because of increased quantity of proteins in serum (overflow proteinuria)**
  3. **Due to low reabsorption at proximal tubule (Fanconi syndrome) (extrarenal proteinurea).**

Other causes:

- **Action of some medications, used in cancer treatment,**
- **Excessive fluid intake (drinking in excess of 4 liters of water per day)**

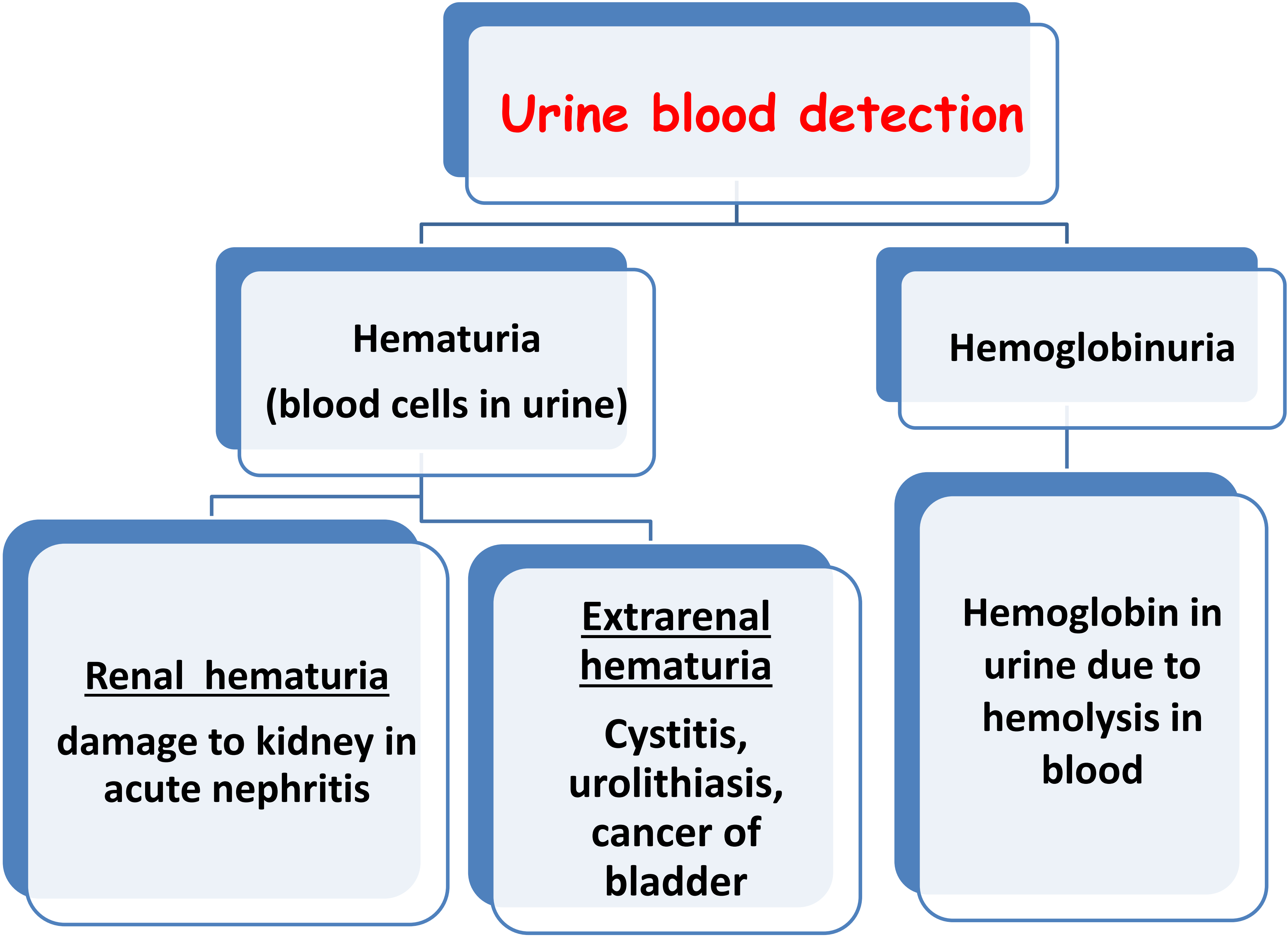
Glucosuria

- **Is the excretion of glucose into the urine**
- **Detectable glucose in urine appears WHEN THE BLOOD GLUCOSE LEVEL EXCEEDS RENAL THRESHOLD (>10 MMOL/L)**

- **Main causes**
  - **Elevated blood glucose level due to different reasons (diabetes mellitus).**
  - **Renal glycosuria due to an intrinsic problem with glucose reabsorption within the kidneys themselves.**

Ketonuria

- Is a medical condition in which ketone bodies are present in the urine
- **Main causes:**
  - Diabetes, renal glycosuria, or glycogen storage disease.
  - Dietary conditions such as starvation, fasting, high protein, or low carbohydrate diets,
  - prolonged vomiting,
  - anorexia.
  - Conditions in which metabolism is increased, such as hyperthyroidism, fever, pregnancy or lactation.


Urine blood detection
Hematuria
(blood cells in urine)
Hemoglobinuria
Renal hematuria
damage to kidney in acute nephritis
Extrarenal hematuria
Cystitis, urolithiasis, cancer of bladder
Hemoglobin in urine due to hemolysis in blood

Bile pigments in urine

- **Breakdown products of normal heme catabolism, caused by the body's clearance of aged red blood cells that contain hemoglobin.**
- **Direct bilirubin is present in the urine both in obstructive and hepatocellular jaundice.**
- **Urobilinogen increases in hepatocellular jaundice.**
- **Stercobilinogen is absent in the urine in obstructive jaundice due to obstruction of the bile duct.**

Clinical Urine Tests

- are various tests of urine for diagnostic purposes.
- Macroscopic Examination *(including naked-eye (gross) examination for color and smell)*
- Microscopic Examination *(presence of RBC, WBC, etc).*
- Cytological Examination *(microbiological culture of urine)*
- Chemical Analysis *(Urine analysis* or *Urinalysis)*

Urine Analysis or Urinalysis

- Fresh Sample = Valid Sample
- The target parameters that can be measured or quantified in urinalysis include naked-eye (gross) examination for COLOR and SMELL plus analysis for many substances and cells, as well as other properties, such as SPECIFIC GRAVITY.
- A part of a urinalysis can be performed by using URINE TEST STRIPS, in which the test results can be read as color changes.

Urine test results should always be interpreted using the reference range of some laboratory tested compounds and enzymes

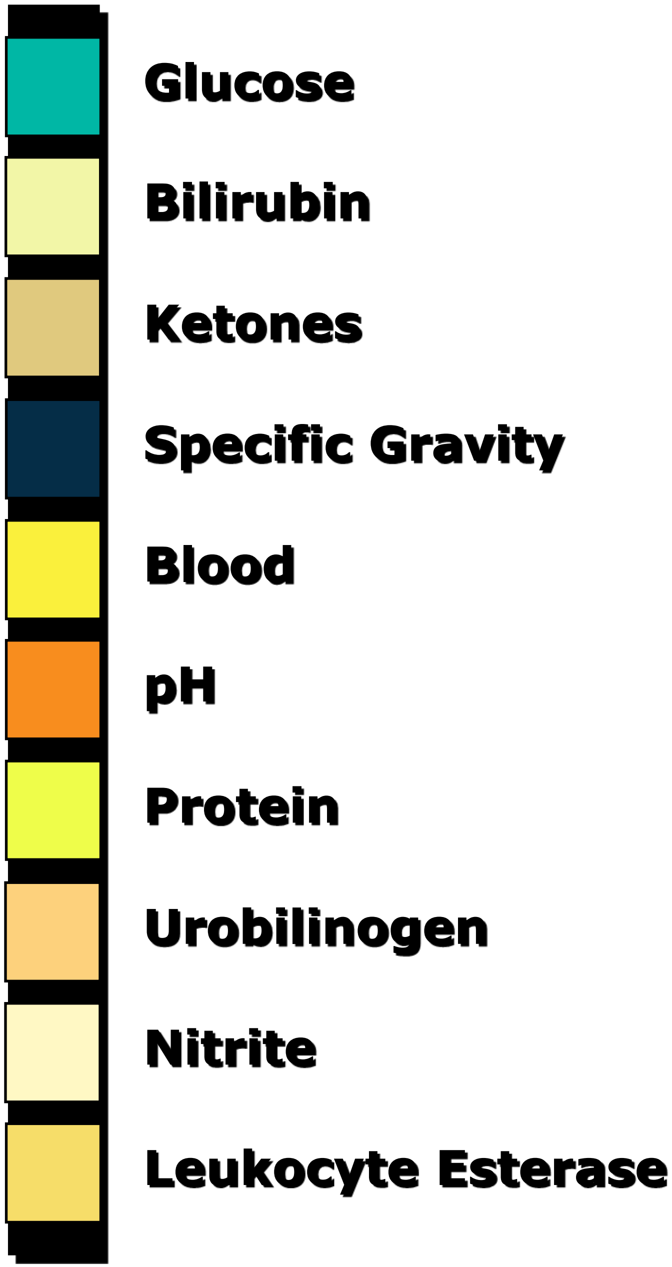
- **Amylase – 28 – 160 g/h.L**
  - comes from pancreas
  - ↑ in acute pancreatitis
- **Uric acid – 1.6 – 6.4 mmol per day**
  - End product of purine metabolism
  - ↑ in leukemia
  - ↓ in gout, nephritis
- **Urea – 333 – 583 mmol per day**
  - End product of protein metabolism
  - ↓ levels is seen in kidney and liver dysfunction
- **Creatinine – 1-2 gram per day (male)**
  0.5 – 1.6 g per day (female)
  - Anhydride form of creatine formed in muscles

Urinalysis test strips

- **A urine sample is tested for several components simultaneously by dipping a test strip into a sample and comparing the reagent areas to a color chart**

- A urine specimen is often collected from a patient entering a hospital and is checked with a **URINE TEST STRIP** that contains bands of different reagents.

- These reangents may react with particular **pathological components, and patogens,** and also include testing of **pH** or **Specific Gravity.**



Case 1

Diabetes

Glucose	++
Bilirubin	Negative
Ketones	Trace
S.G.	1.015
Blood	Negative
pH	6.0
Protein	Negative
Urobilinogen	1.0 mg/dL
Nitrite	Negative
L.E.	Negative

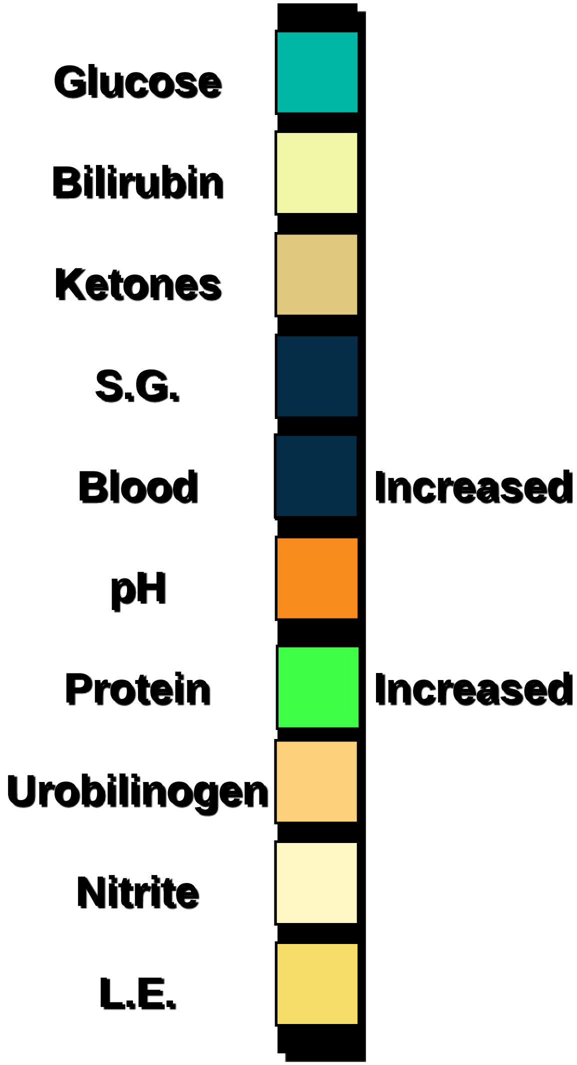
A 27-year old woman enters a hospital with severe abdominal pain.

Physical characteristics: clear-yellow.

Microscopic: Not performed.

Case 2

Common Findings in: Acute Glomerulonephritis

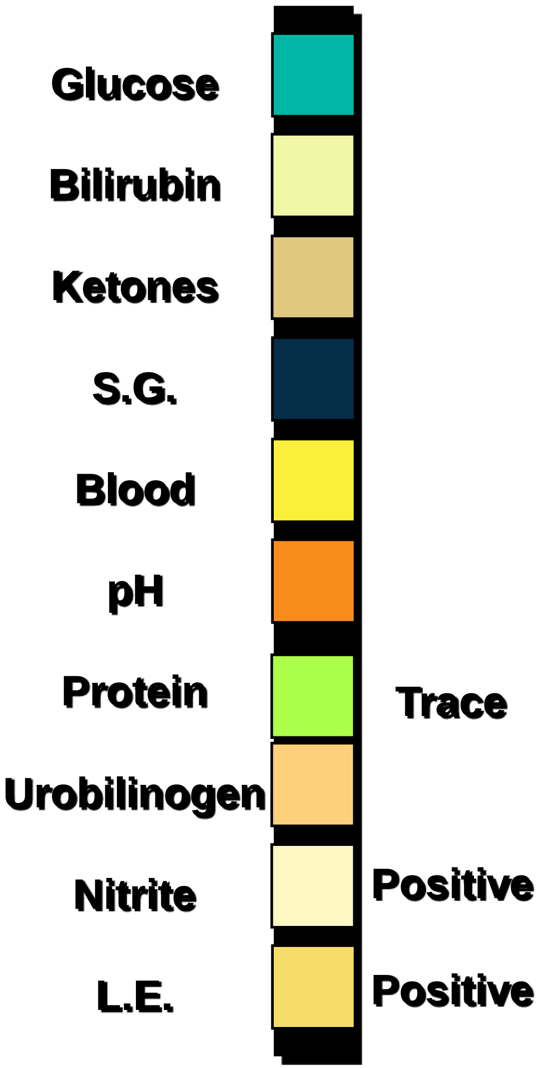


Microscopic:

- Erythrocytes (dysmorphic)
- Erythrocyte casts
- Mixed cellular casts

Case 3

Common Findings in: Acute Pyelonephritis



Microscopic:

- Bacteria
- Leukocytes
- Leukocyte, granular, and waxy casts
- Renal tubular epithelial cell casts