CREATININE ESTIMATION AND CREATININE CLEARANCE TEST

OBJECTIVES

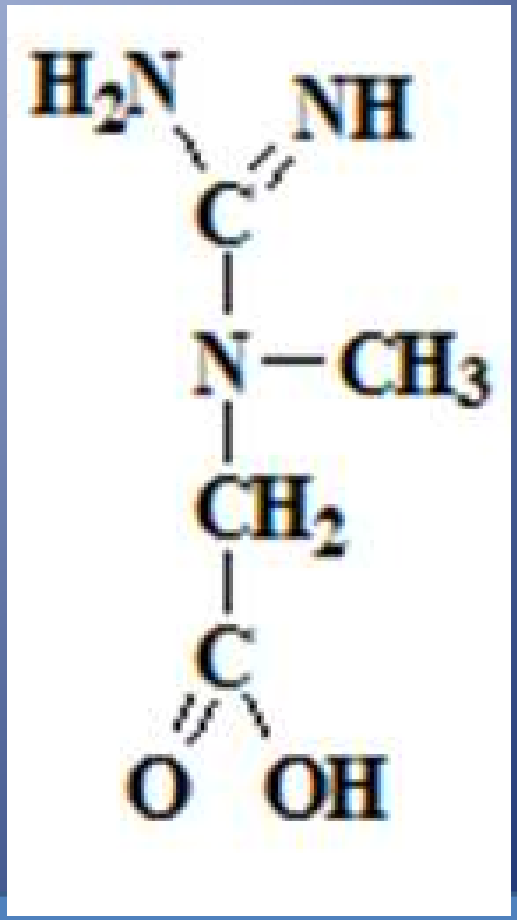
1. To estimate creatinine in serum and urine.
2. To calculate creatinine clearance test value.
3. To asses renal function, i.e. to asses the glomerular filtration rate.

What's Cratine and Creatinine…?

- Creatine and creatinine are not the same substance!
- Creatine is found in the muscles…….
- **Creatinine** is a break-down product (a waste product) of **creatine phosphate and creatine** in **muscles**, and is usually produced at a fairly constant rate by the body (depending on muscle mass).

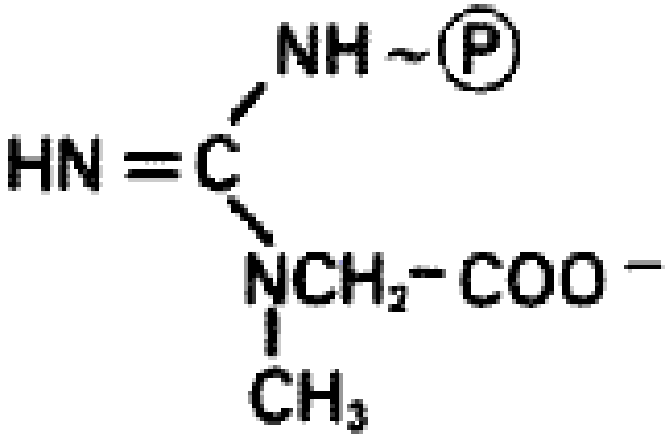
Definition of Creatine

1. The creatine is an amino acid that does not found in proteins.
2. Creatine is a nitrogenous organic acid.



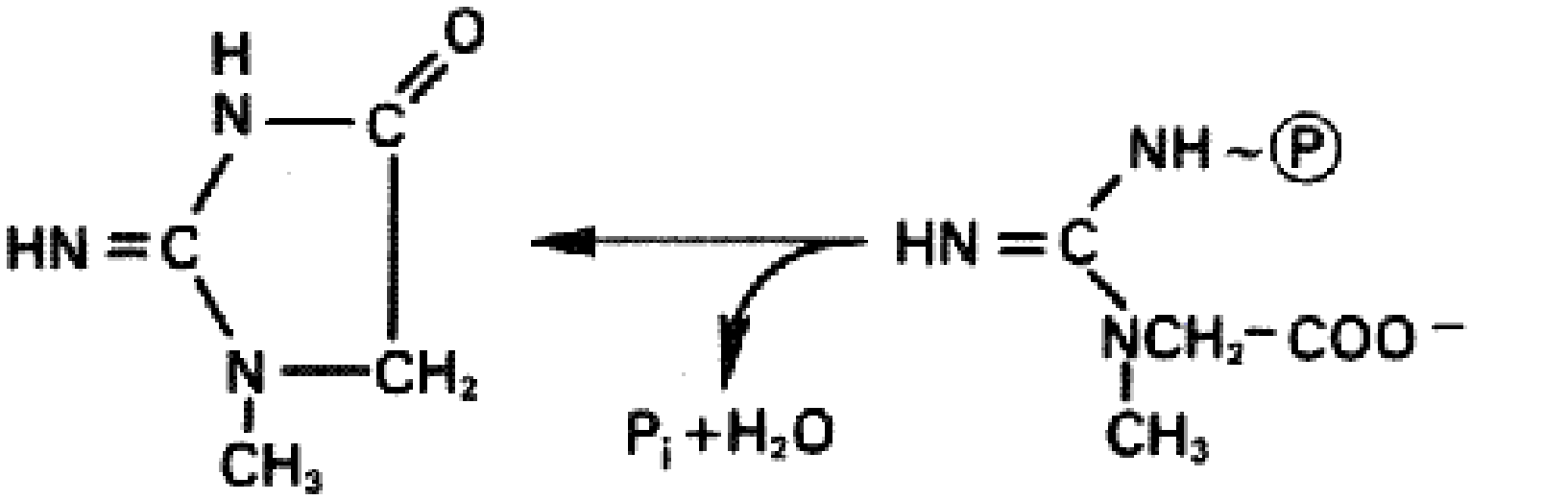
Structure of Creatine

Structure of Creatine Phosphate



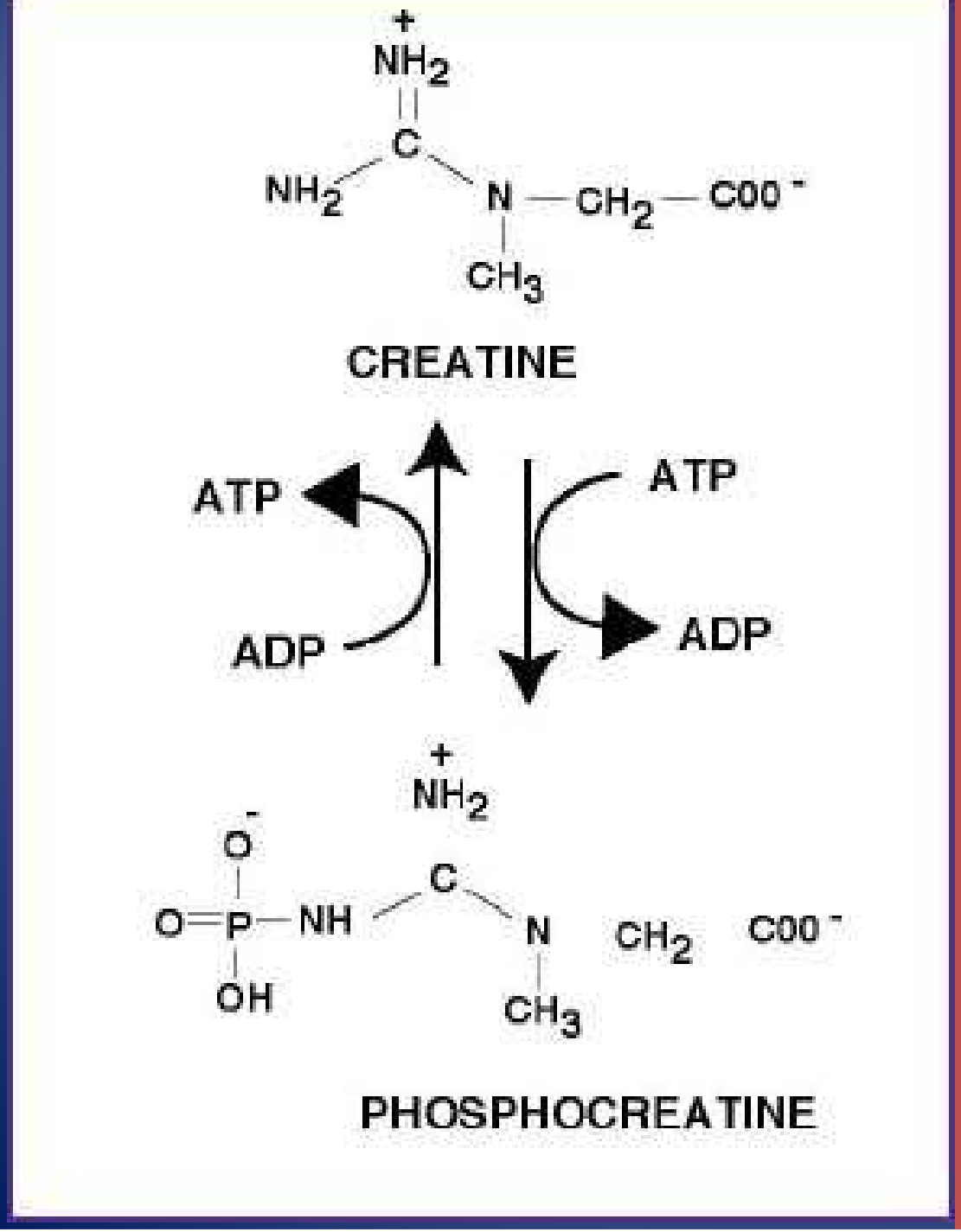
Creatine ~ phosphate

Formation of Creatinine from Creatine Phosphate



What's the Relationship between Creatine and Creatinephosphate?

- **Creatine** and **creatine phosphate** exist in a reversible equilibrium in skeletal muscle.
- In skeletal muscle, approximately one-fourth of creatine exists as free creatine and three-fourth exists as creatine phosphate.

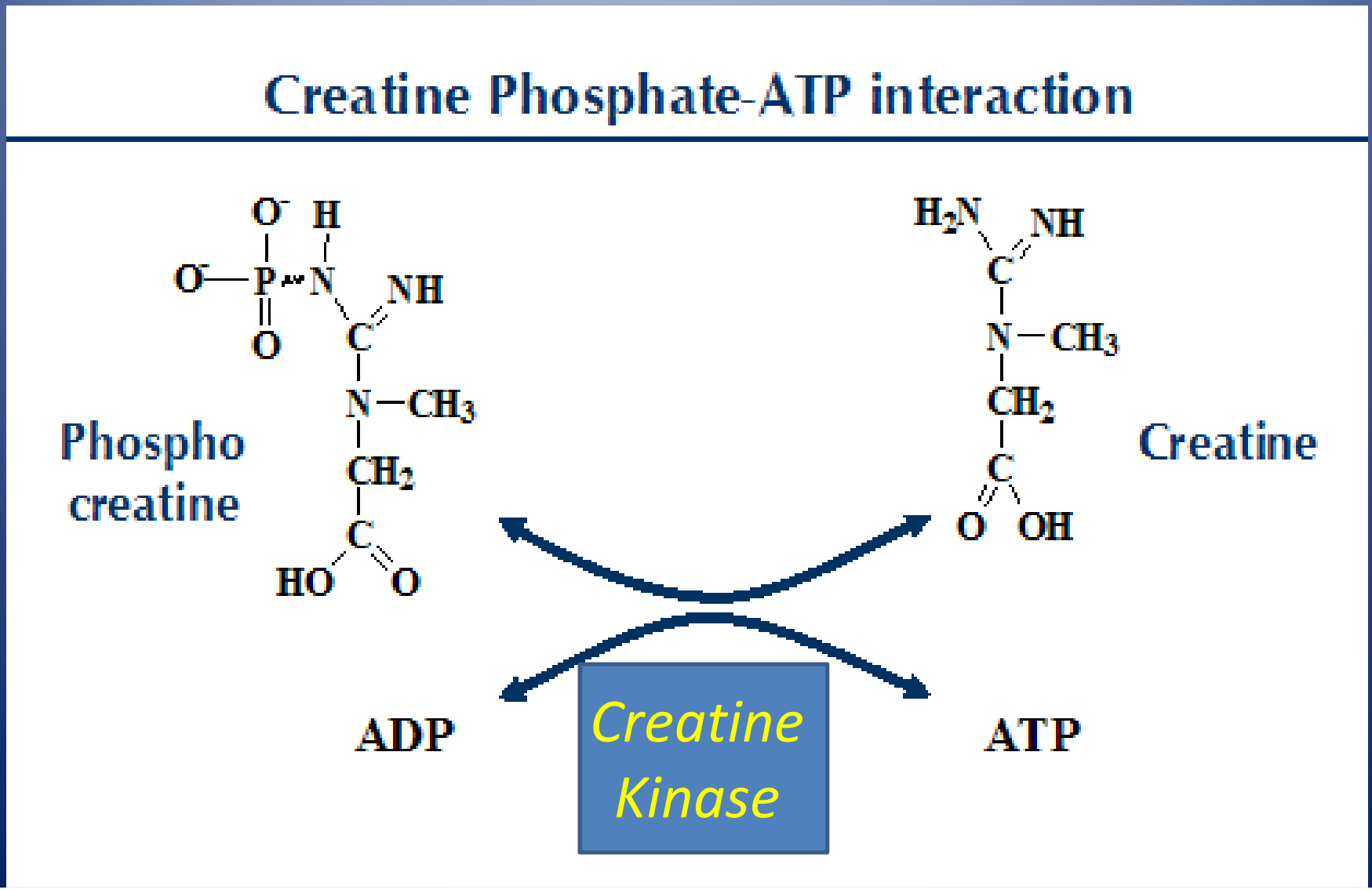


The Reversible Equilibrium between Creatine and Phosphcreatine

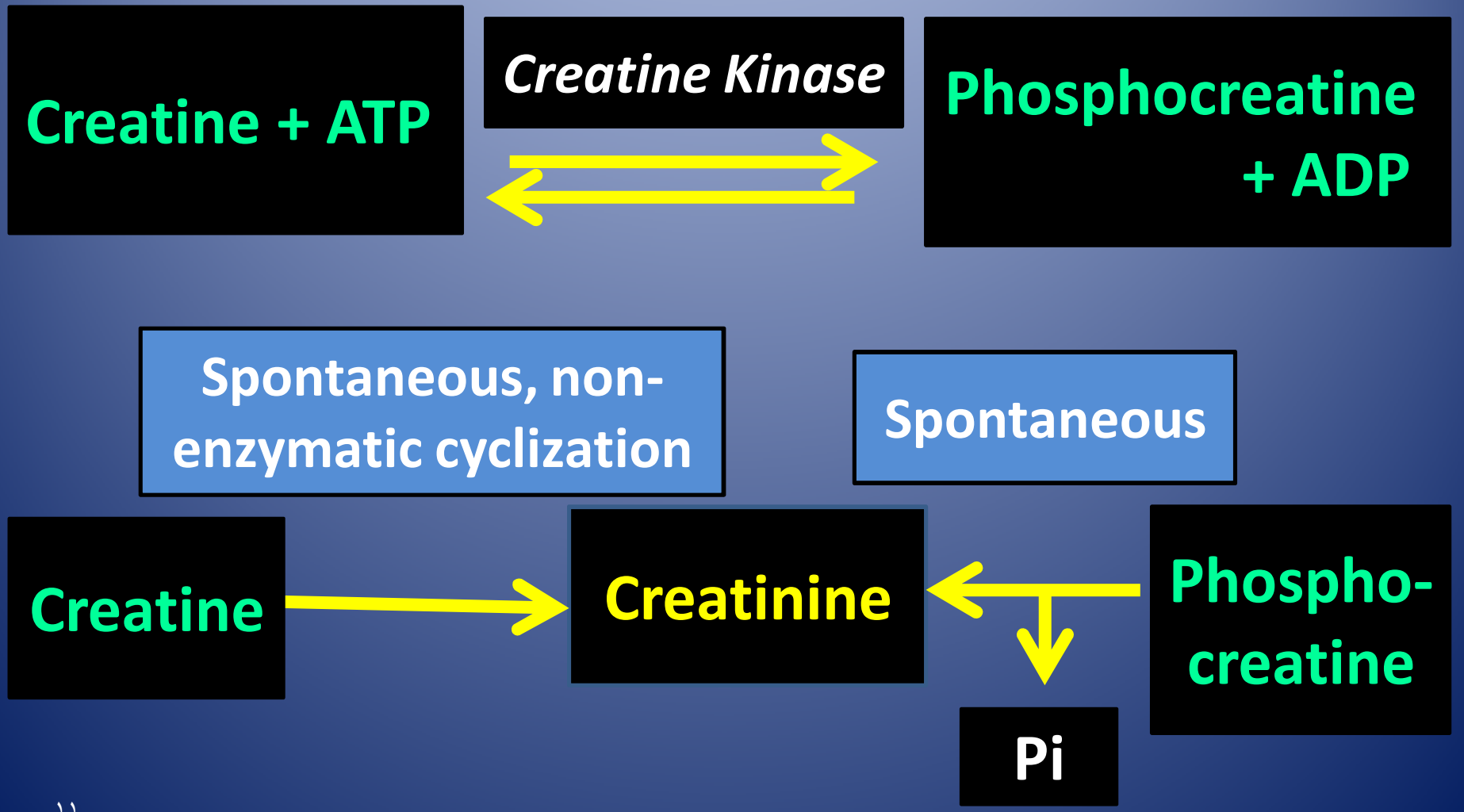
Creatine Phosphate is a Compound with High-Energy Phosphate Bond

- ***Creatine can be charged with energy:***
  The enzyme creatine kinase (CK) which transfers the high-energy phosphate group from ATP to creatine to make creatine phosphate, a compound with high-energy phosphate bond.

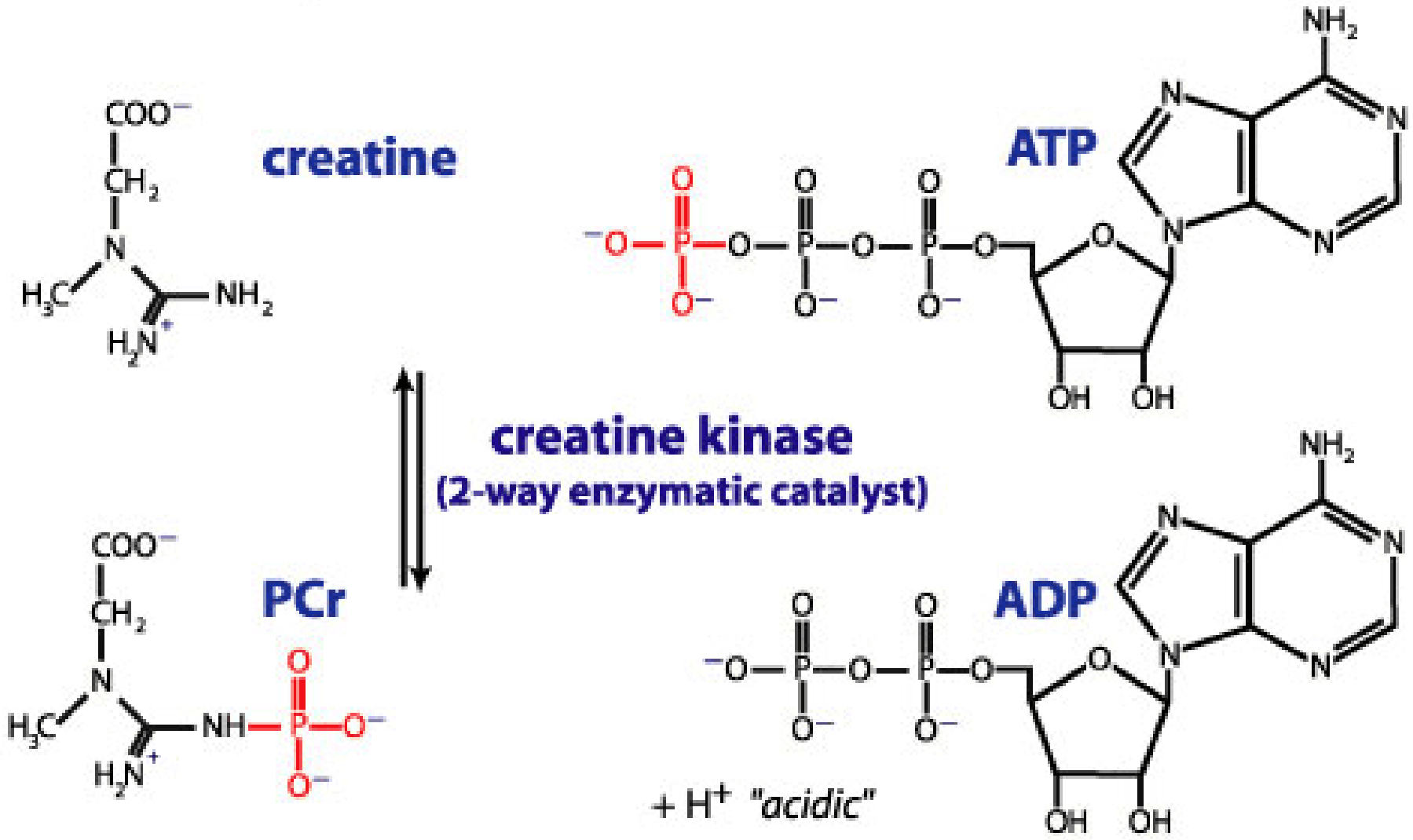
The Reversible Equilibrium between Creatine and Phosphocreatine



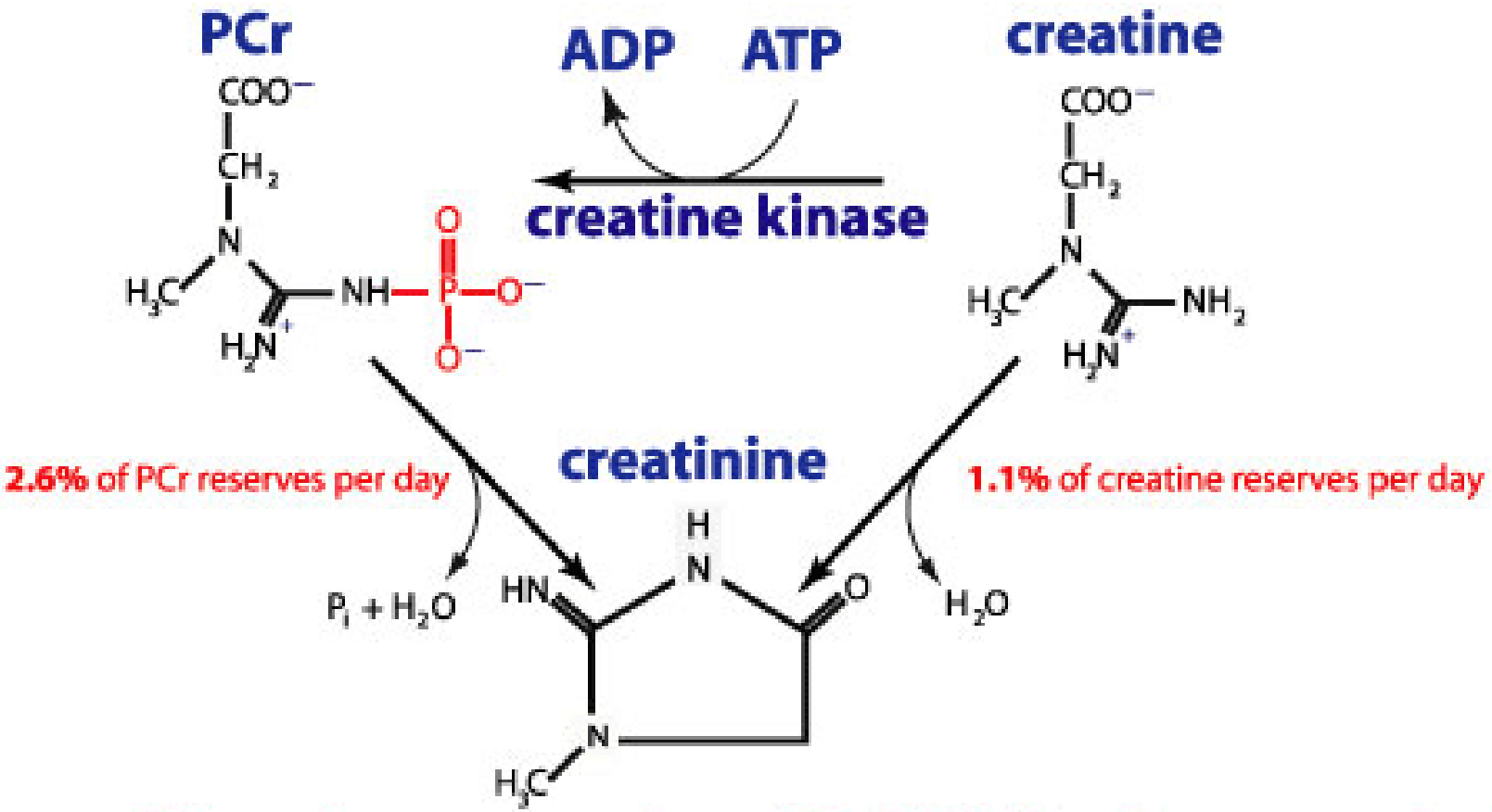
Formation of Creatinine



Enzymatic Interconversion of PCr and ATP



Spontaneous One-Way Conversion of PCr and Creatine



The rate of formation of creatinine from phosphocreatine is twice the rate of formation of creatinine from free creatine.

Creatine Phosphate is a Source of Energy During Exercise….

- Creatinephosphate, by reversal of the above reaction, plays an important role in recharging ADP to ATP during severe ***anaerobic exercise***.
- The reaction contributes to ATP resynthesis for about 10 to 20 seconds of maximal exercise.

Why Creatine Phosphate is Important for the Body..?

- Creatine phosphate functions as a **'battery'** that stores the energy at excess ATP.
- ***Therefore it has a major importance for energy production in muscles*.**

We Know that Creatine is Found in the Muscles ….but Where is it Synthesized ?

1. **Endogenouse Source**

- **CREATINE SYNTHESIS IN DIFFERENT TISSUES OF THE BODY**: Creatine is synthesized primarily by the liver, kidneys, and pancreas at a rate of 1 to 2 g/day.

2. **Exogenouse Source** : An additional 1 to 2 g/day is obtained in the diet, mainly from fish and meats.

What is the Fate of Creatinine that was Produced from Break Down of Creatine Phosphate during Anaerobic Exercise..?

❑ CREATININE EXCRETION

- **The creatinine is a waste product of creatine phosphate and *it will be excreted*** *by the kidney in the urine at a rate of 1 to 2 g/day.*

Creatinine Metabolism

Approximately 2% of the body's creatine is converted to creatinine every day

Creatinine is transported through the bloodstream to the kidneys

The kidneys filter out most of the creatinine and excrete it in the urine.


What Happens to
Creatinine Level (in
Both Blood and
Urine) if the Patient
Has Renal Failure….?

The Diagnostic Function of Creatinine

If the kidneys are damaged or impaired and cannot work normally

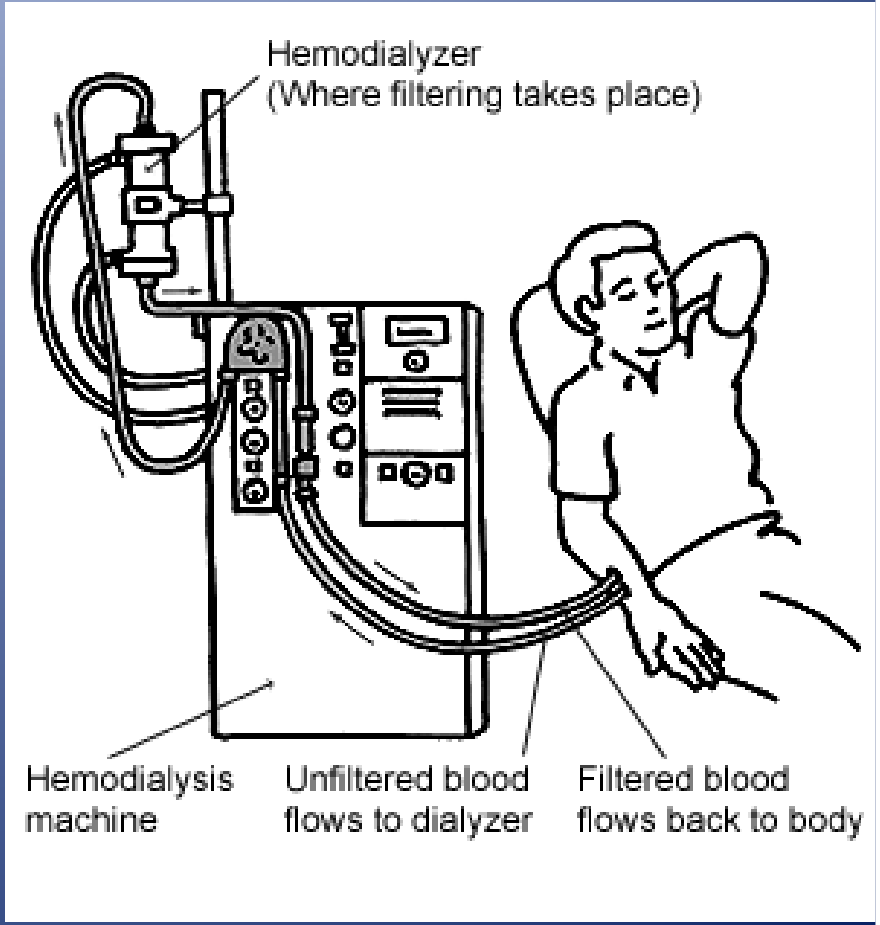
The amount of creatinine in urine goes down while its level in blood goes up.

Creatinine has been found to be a fairly reliable indicator of kidney function.

Serum creatinine level is an important diagnostic tool to asses renal function

How is Creatinine Used to Monitor Renal Function…?

- **Serum creatinine :**
  (creatinine level in the blood)
- In renal failure the kidney will not be able to excrete creatinine in urine leading to an elevation in serum creatinine level.



Levels of Creatinine in the Blood Depends Mainly on Renal Function….but…

- Is there other factors may affect creatinine level in the blood…?

Serum Creatinine may be Affected Partly by….

1. **The amount of muscle tissue you have.**
   Men tend to have higher levels of blood creatinine because they have more ***skeletal muscle tissues*** than women.
2. **Protein in diet** . Vegetarians have been shown to have lower creatinine levels in blood.

How is Creatinine Used to Monitor Renal Function....?

1. Creatinine Clearance Test:

- Measures ***how well creatinine is removed from blood by kidneys*** [which reflects glomerular filtration rate (GFR)].

How is Creatinine Used to Monitor Renal Function....?....cont.

1. Creatinine Clearance Test

- A ***Creatinine Clearance Test*** gives better information than a blood creatinine test on how well your kidneys are working (as serum createnine may be affected by muscle bulk).

The Glomerular Filtration Rate

- GFR is the rate at which blood is filtered through all of the glomeruli (the plaural of glomerulus)

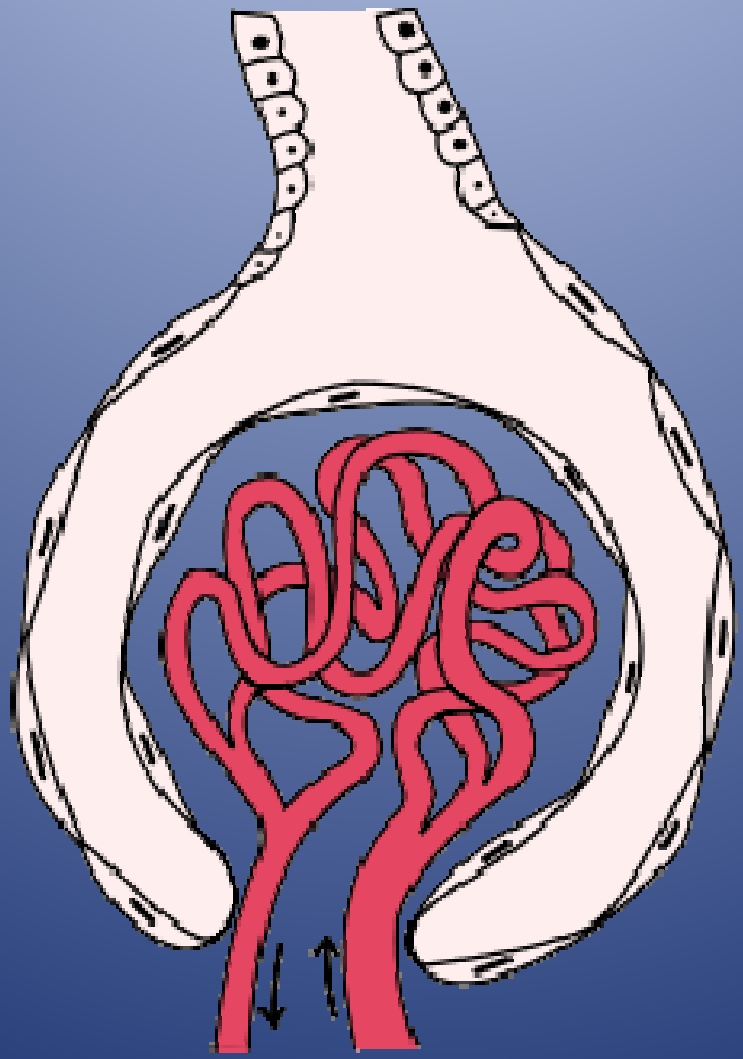
or

- GFR is the measure of fluid filtered from the renal glomerular capillaries into the Bowman's capsule per unit time.

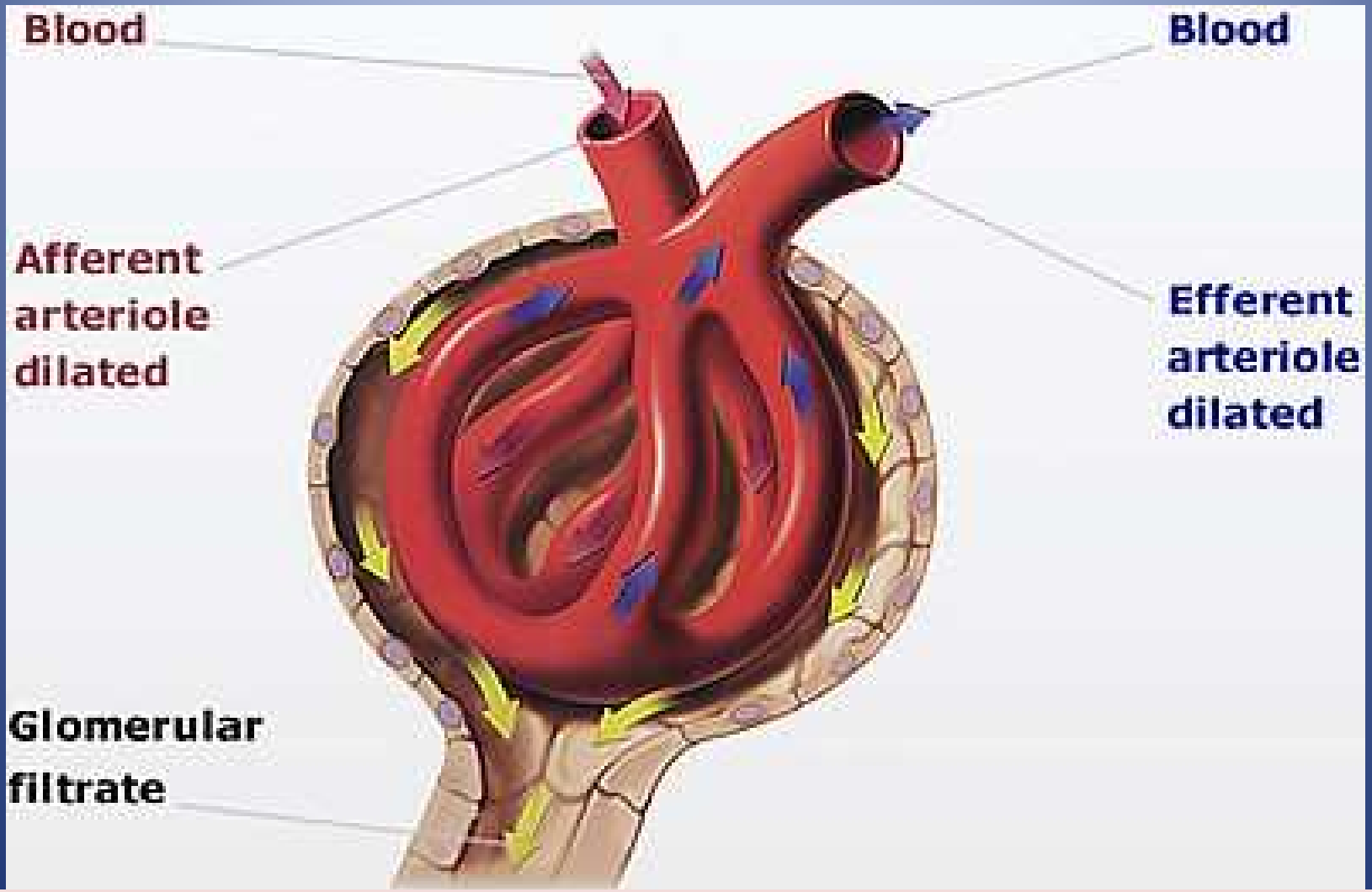
GFR is often used to determine renal function

WHAT IS THE GLOMERULUS ?

Glomerulus Structure



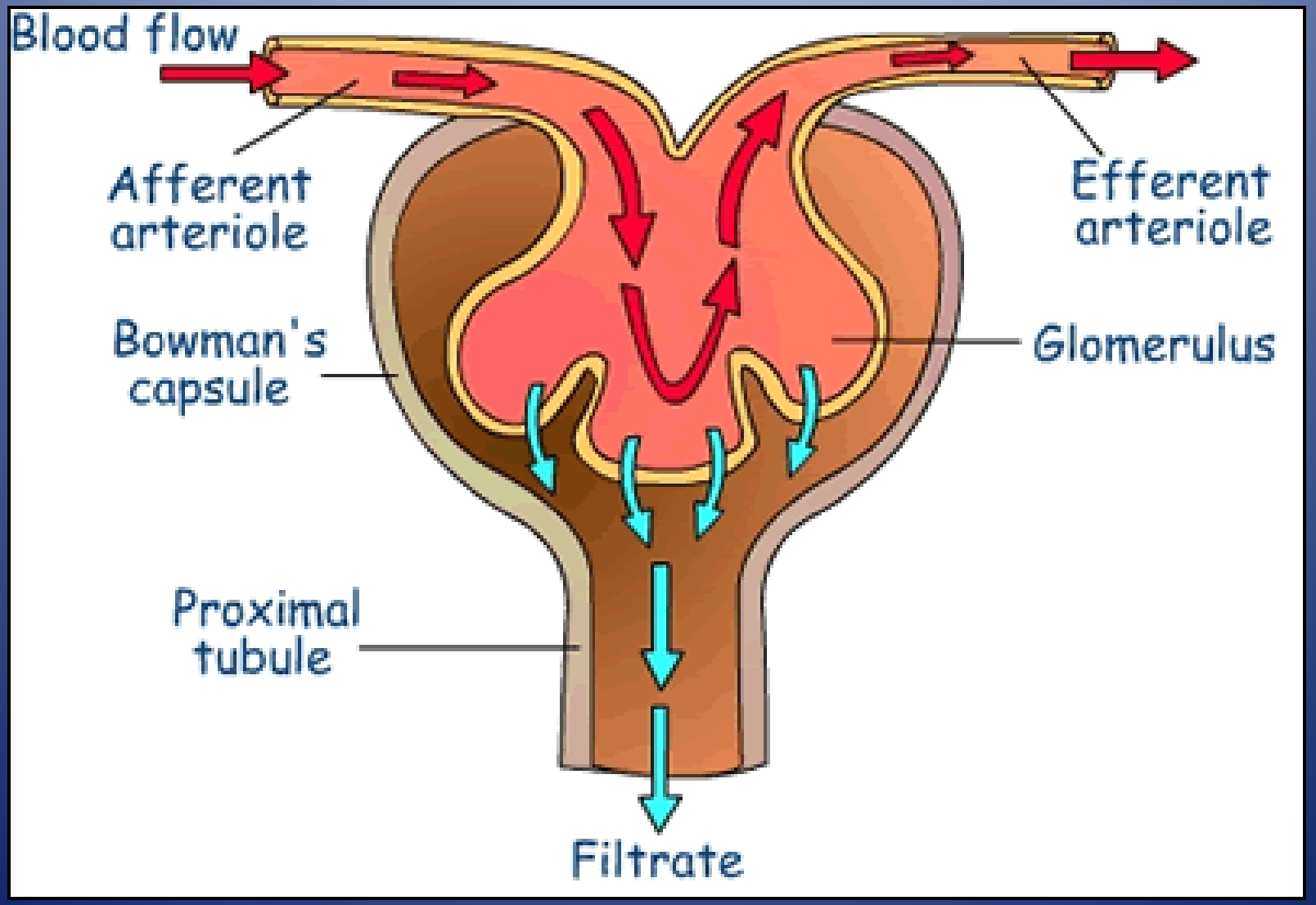
Structure and Function of Glomerulus



1. A glomerulus is one of the small units in the kidney made up of capillaries and nerve fibers or it is a capillary tuft surrounded by Bowman's capsule in nephrons of the kidney.
2. It is the structure where blood filtration takes place.

Structure and Function of Glumerulus

Glomerular Filtration Rate



How is Creatinine Used to Monitor Renal Function? ……cont.

- In renal failure, the filtering (GFR) of the kidney is deficient, *blood levels of some substances will rise and urine levels will decrease.*
- **Creatinine Clearance.** Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which reflects the GFR.

How to do Creatinine Clearance Test ?

❑ The Principle Protocol of Creatinine Clearance Test :

- A creatinine clearance test is done on both a blood sample and on a sample of urine collected over 24 hours (24-hour urine samples).


Are there any Substances Used to Assess GFR other than Creatinine ?

Different Clearance Tests Used to Assess Renal Function

- ***Kidney function tests (Clearance tests):***

  is a collective term for a variety of individual tests and procedures that can be done to evaluate how well the kidneys are functioning.

- **There are 3 major clearance tests:**

1. **Creatinine clearance test.**
2. **Urea clearance test.**
3. **Inulin clearance.**

2- Urea Clearance Test

- ***What's urea ….?***

- **Urea is a:**

1. Waste product of protein; it is created by protein catabolism
2. Excreted in the urine.

2- Urea Clearance Test ….cont.

- Urea clearance test is a clinical test for renal function determined by the clearance of urea from the plasma by the kidney each minute.

How to do Urea Clearance Test ?...Method...

- **Urea clearance test** in order to determine the amount of urea that is filtered, or cleared, by the kidneys into the urine we require the following **:**

1. A blood sample to measure the amount of urea in the bloodstream
2. Two urine specimens, collected one hour apart.

3- Inulin Clearance Test

❑ What's Inulin ?

- ***Inulin*** **is the most accurate substance, after creatinine**, to asses renal function because:

1. it is a small, inert polysaccharide molecule that readily passes through the glomeruli into the urine.
2. It is not reabsorbed by the renal tubules.

The Preference of Creatinine Clearance Test in Evaluating Kidney Function ....cont.

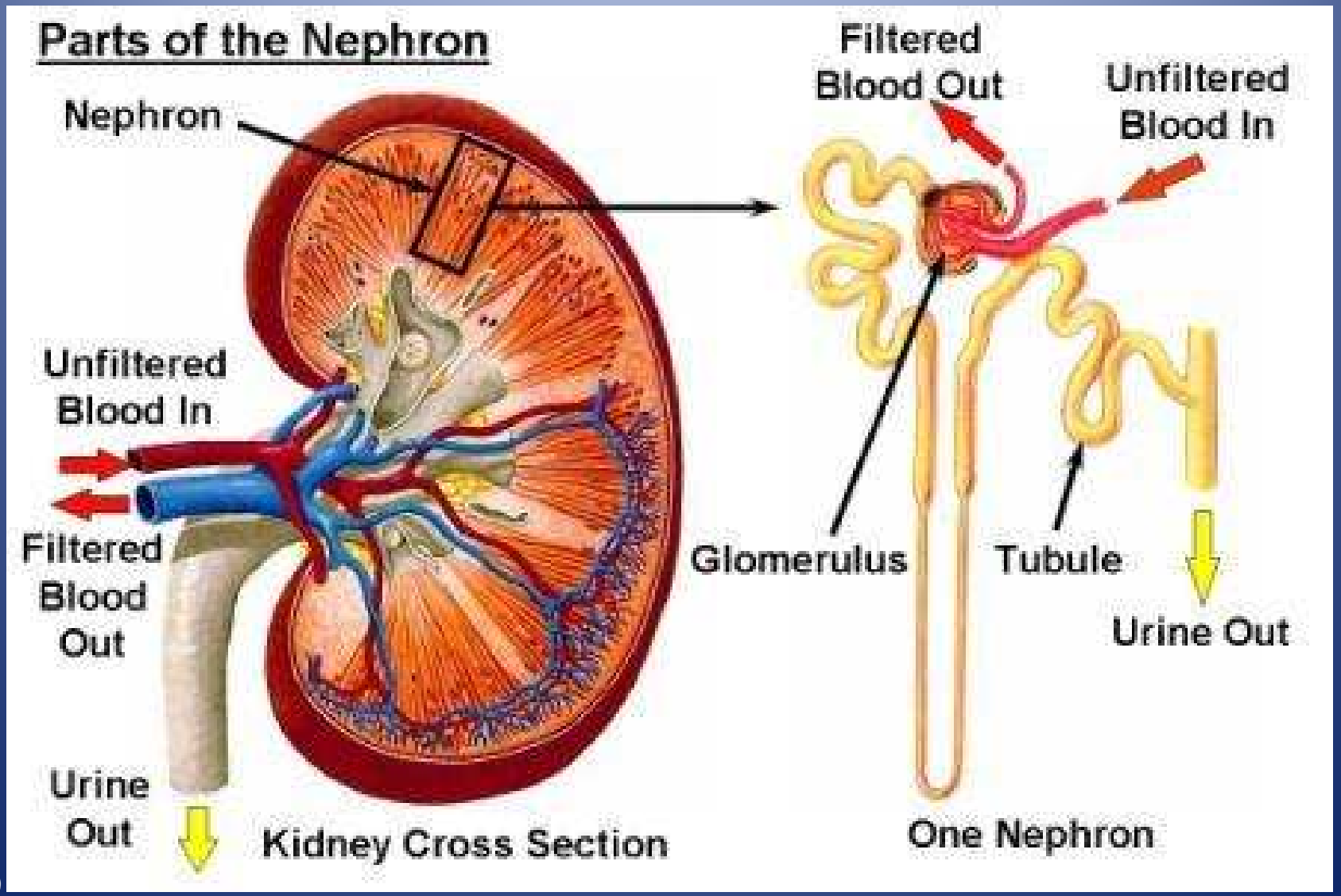
- Creatinine clearance is the preferred, most common procedure used to assess renal function.

The Preference of Creatinine Clearance Test in Evaluating Kidney Function ….cont.

- **Creatinine clearance is preferred because:**

1. It is a normal constituent of blood and no infusion is needed unlike inulin.
2. Moreover it is not reabsorbed by the tubules as in the case of urea.

Glomerular Filtration Rate



Clearance Tests

❑ Clearance Tests

- One of the most important kidney function tests, it is; the amount of plasma (in ml) that is cleared from a certain substance by the kidneys per minute.

Clearance = UV/P

- U = concentration of the substance in urine.
- P = concentration of the same substance in plasma
- V = volume of urine (ml/min)

High Levels of Creatine

- **High Levels of Creatine in blood and urine are caused by** :

1. Break down of muscle cells.
2. Starvation and fever.
3. Diabetes mellitus.
4. Hyperthyroidism.

Principle

❑ Determination of Creatinine

- The methods most widely used today are based on the ***Jaffe reaction***. This reaction occurs between creatinine and the picrate ion formed in alkaline medium (sodium picrate); a red-orange solution develops.

Principle…..cont.

Creatinine + Picric acid

Yellow color

NaOH (Alkaline medium)

Creatinine Picrate

Orange color

Measured colorimetrically at 520 nm

Materials

1. Creatinine stock standard: 150 mg creatinine in 100 ml water (1.5 mg/ml)
2. Creatinine working standard for serum (3mg/dl): dilute 10 ml of stock and increase the volume up to 500 ml with water.
3. Creatinine working standard for urine (0.75 mg/dl): dilute 50 ml of stock and bring the volume up to 200 ml with water.
4. Serum and urine samples.
5. NaOH (2.5 M).
6. Picric acid.

- **Method**

1. Serum Creatinine :

	Standard (A,B)	Test (C,D)	Blank (B)
Serum	-	0.5 ml	-
Water	1.5 ml	1.5 ml	2 ml
Standard for serum	0.5 ml	-	-
Picric acid	6 ml	6 ml	6 ml

1. Mix well
2. Add 0.4 ml of 2.5 M NaOH
3. Allow to stand for 20 minutes
4. Read the absorbance against the blank at 520 nm

Method ……cont.

2) Urinary Creatinine

	Standard (A,B)	Test (C,D)	Blank (B)
Urine	-	0.5 ml	-
Water	1.5 ml	1.5 ml	2 ml
Standard for urine	0.5 ml	-	-
Picric acid	6 ml	6 ml	6 ml

Method ……cont.

2) Urinary Creatinine

5- mix well

6- Add 0.4 ml of 2.5 M NaOH

7- Allow to stand for 20 minutes

8- Read the absorbance against the blank at 520 nm

Calculations

- **Serum creatinine** = $\frac{\text{Abs of test}}{\text{Abs of std}}$ X concentration of **standard**
- **Urinary creatinine** = $\frac{\text{Abs of test}}{\text{Abs of std}}$ X concentration of **standard**
- **Creatinine clearance** = $\frac{\text{Urinary creatinine}}{\text{Serum creatinine}}$ X Volume of urine ml/min/1.73 m^2

❑ **Normal creatinine clearance: 100-130 ml/min/1.73m^2.**