SERUM CREATININE:

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Out lines

Biochemistry & physiology

Sample collection

Analytical methods

Reference intervals & biological variability

What is creatine & creatinine

Creatine & creatinine are not same substances

Creatine is found in muscle .....

Creatinine is break down product (waste product) of creatine phosphate & creatine in muscle
Amount of creatinine produced each day is fairly constant & related to muscle mass

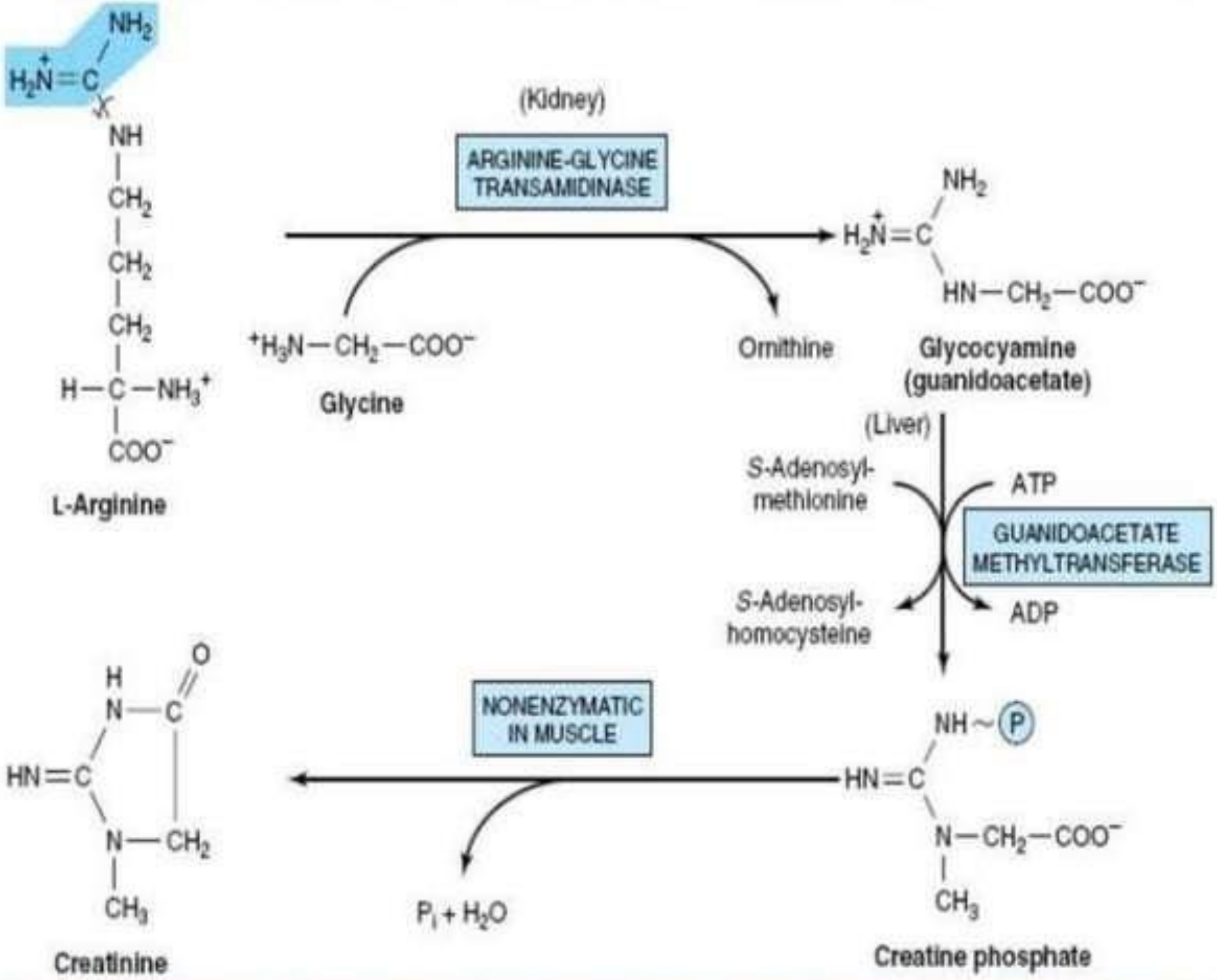
Steps in formation of creatine

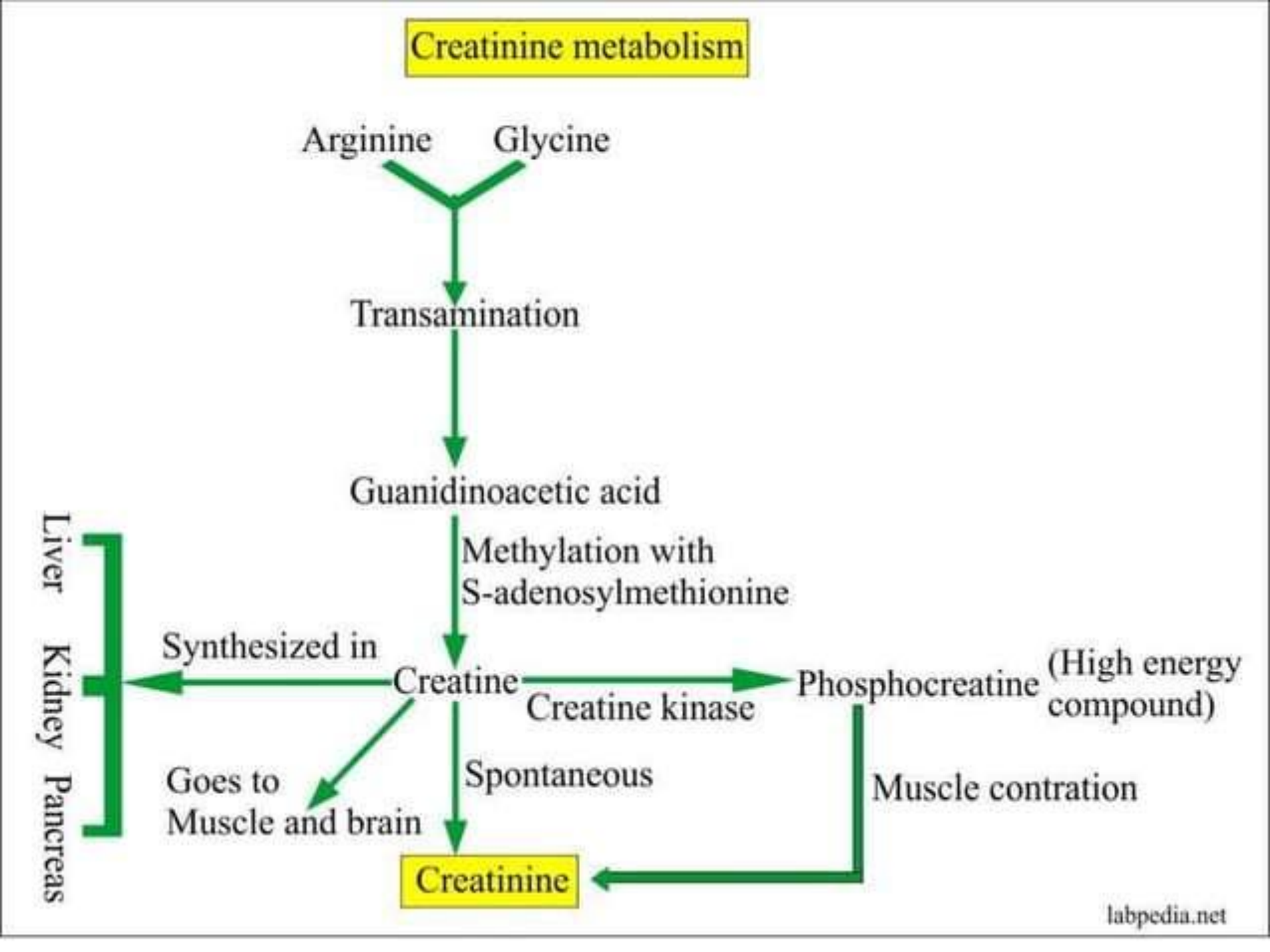
Creatine is formed from three amino acids
1-Glycine 2-Arginine 3-Methionine

Site of biosynthesis

Step1- kidneys Step2- liver

a-Trans amidation-occurs in kidney
b-Transmethylation – occurs in liver
c-phosphorylation-occurs in Muscles


(Kidney)
ARGININE-GLYCINE TRANSAMIDINASE
L-Arginine
Glycine
Ornithine
Glycocyamine (guanidoacetate)
(Liver)
S-Adenosyl-methionine
ATP
GUANIDOACETATE METHYLTRANSFERASE
S-Adenosyl-homocysteine
ADP
NONENZYMATIC IN MUSCLE
Creatinine
Creatine phosphate
$P_i + H_2O$


Creatinine metabolism
Arginine
Glycine
Transamination
Guanidinoacetic acid
Methylation with
S-adenosylmethionine
Liver
Kidney
Pancreas
Synthesized in
Creatine
Phosphocreatine
(High energy
compound)
Creatine kinase
Goes to
Muscle and brain
Spontaneous
Muscle contration
Creatinine
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Distribution of body creatine

From liver ,transported to other tissues

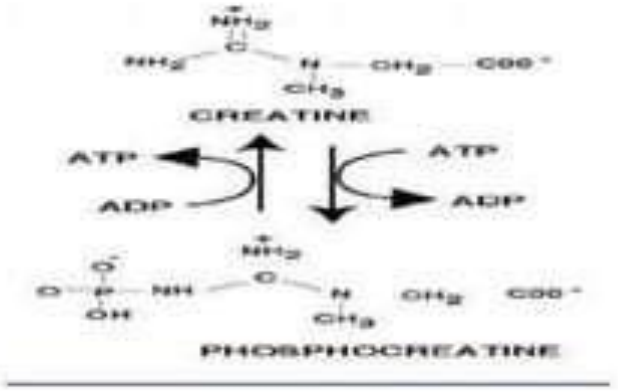
98% is present in skeletal & heart muscles

In muscle converted into high energy source creatinine phosphate

Relationship between creatine & phosphocreatine

Creatine & creatinine phosphate exits in a ***reversible equilibrium*** skeletal muscle

In skeletal muscle approximately *one fourth* of creatine exists as free creatine & *three fourth* as creatinine phosphate



Creatine phosphate

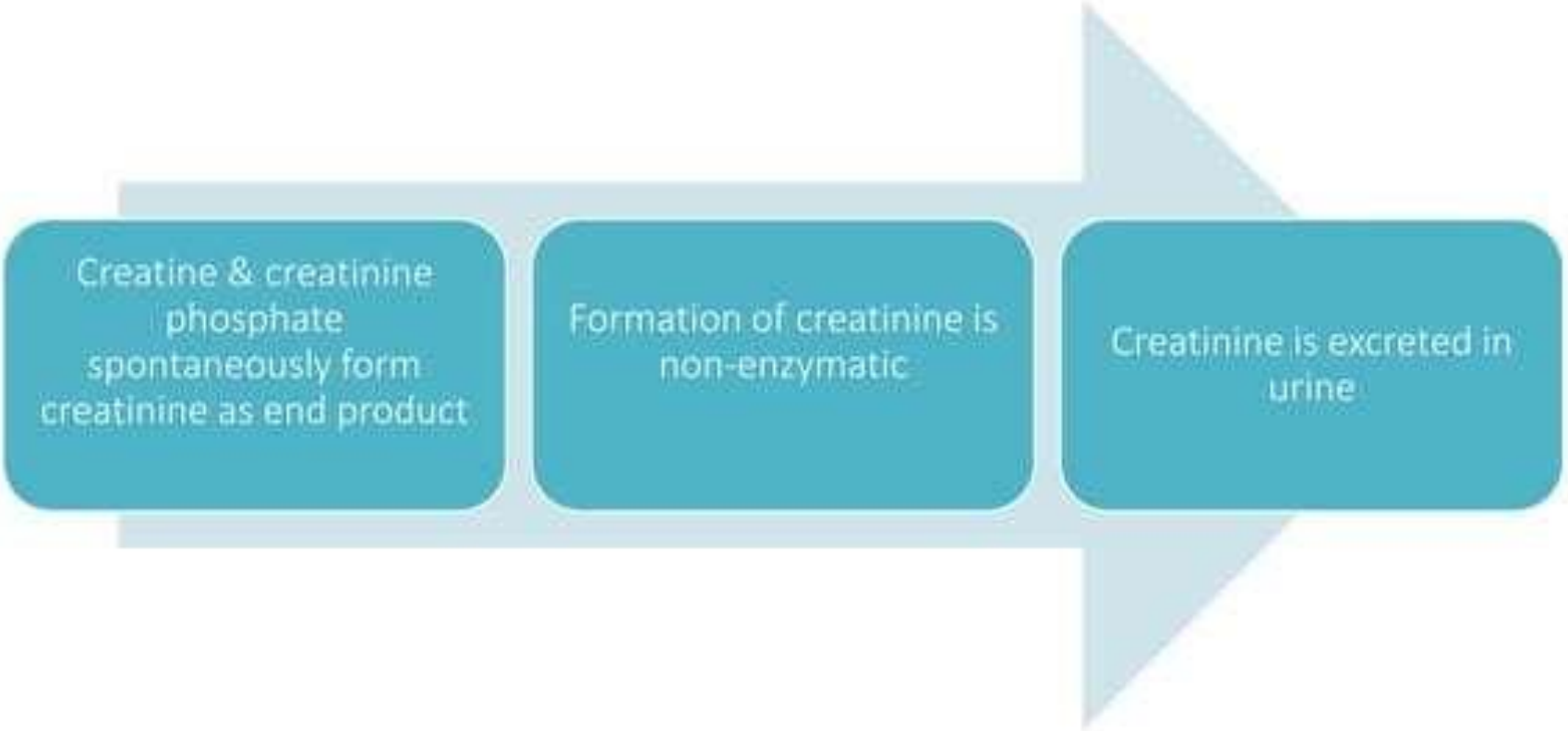
High energy phosphate compound .

Act as storage form of energy in muscle

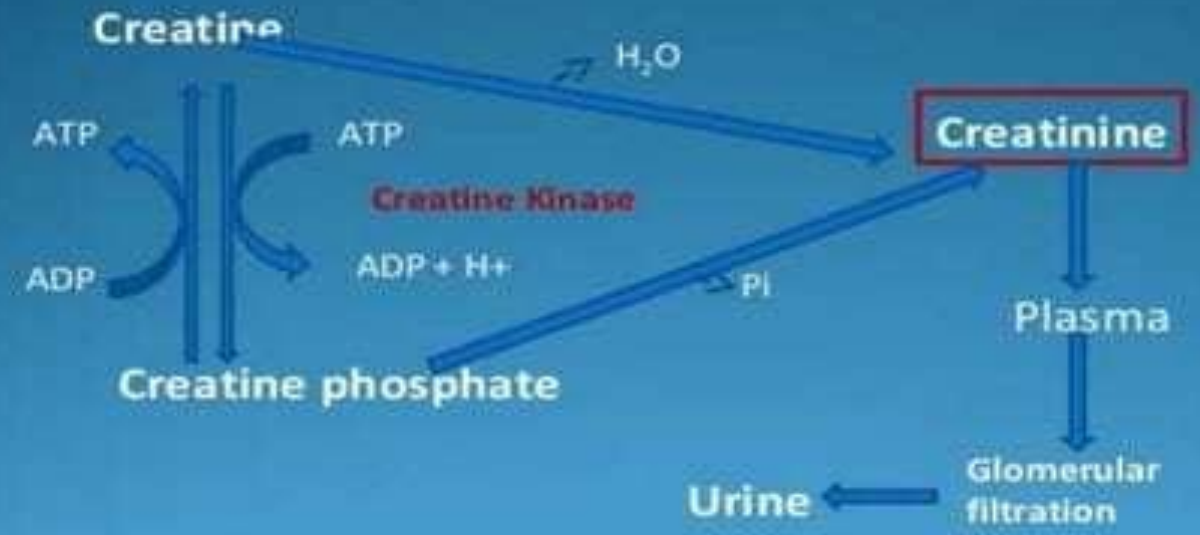
Provides a small, but ready source of energy during first few minutes of energy contraction

The amount of creatine phosphate present in body is directly proportional to muscle mass

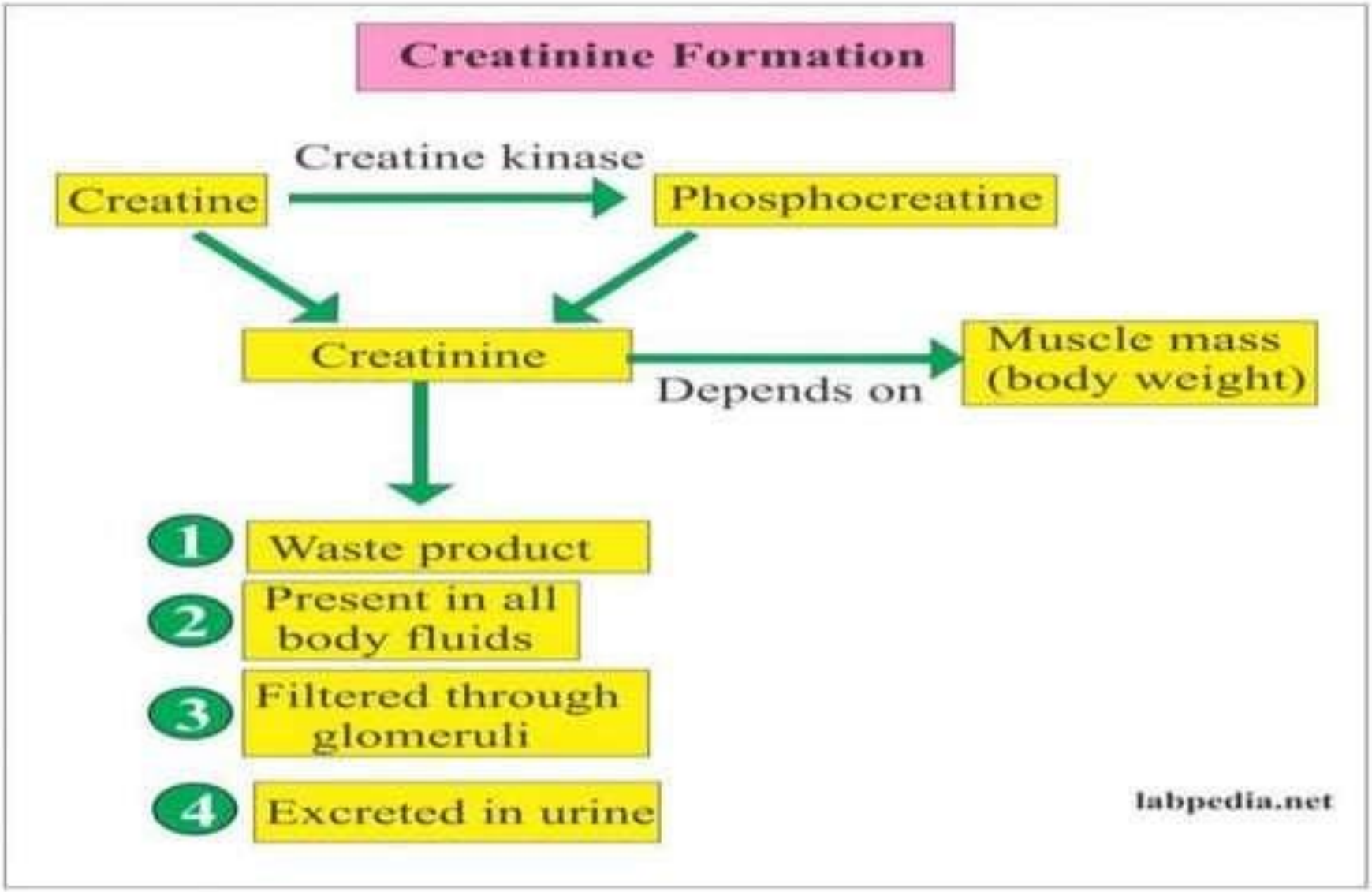
Creatine degradation



Creatine Degradation



Creatinine :


Creatinine Formation
Creatine kinase
Creatine
Phosphocreatine
Creatinine
Depends on
Muscle mass (body weight)
1 Waste product
2 Present in all body fluids
3 Filtered through glomeruli
4 Excreted in urine
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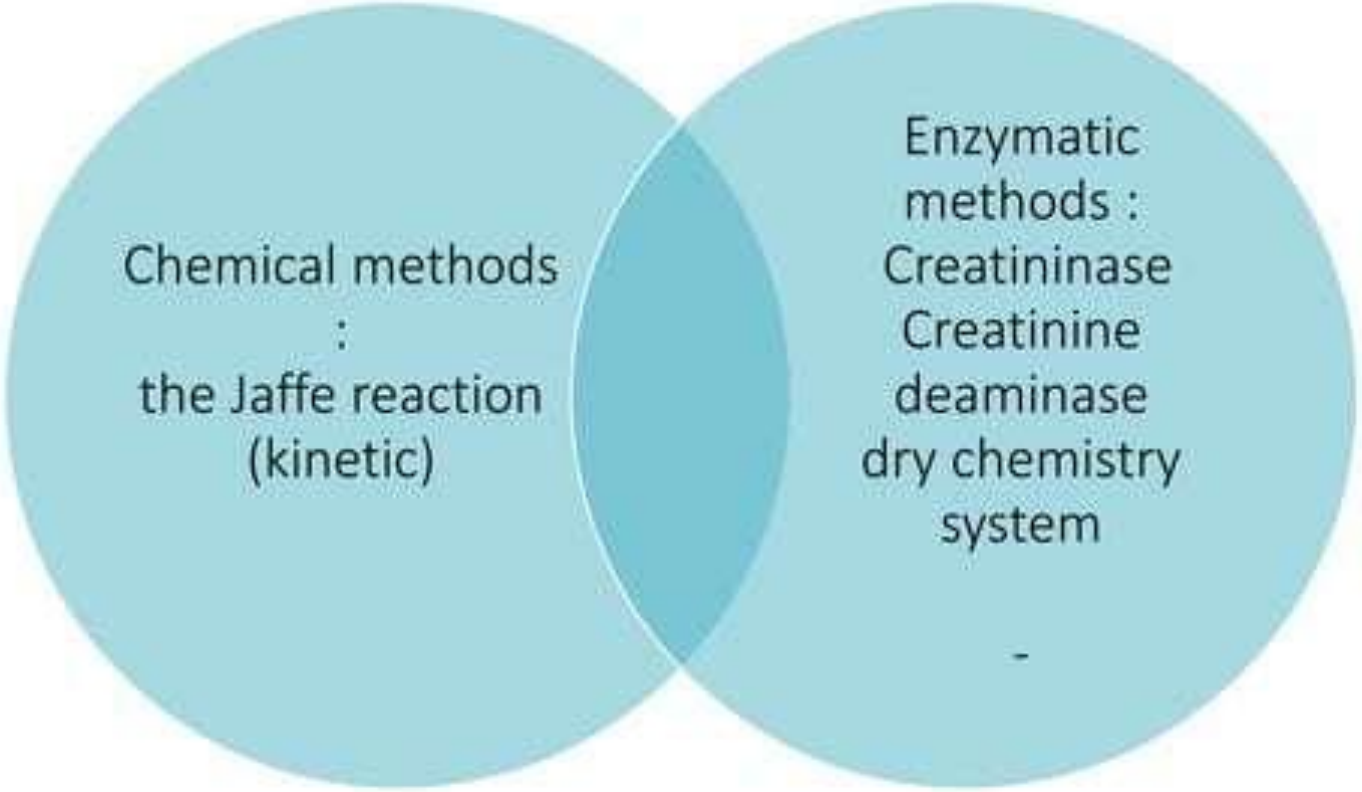
Sample collection & storage :

Serum, plasma (lithium heparin) and urine are the recommended sample types for this assay.

Separated serum and plasma specimens may be stored for up to 24 hours at room temperature (18–26°C) or for up to 7 days at 2–8°C or stored frozen for up to 3 months at -20°C or colder.

Urine specimens (random or 24-hour collections) are stable for up to 4 days at 2–8°C or are frozen for storage longer than 4 days.

Analytical methods



Enzymatic method : Creatininase

This enzymes catalyzes conversion of creatinine to creatine , creatine is then detected with series of enzymes mediated reaction involving creatine kinase ,pyruvate kinase , & lactate dehydrogenase ,with monitoring of decrease in absorbance of at 340nm.

Having poor precision, sensitivity, & high cost of reagent, high incubation period 30minutes .

Enzymatic method :

$$\text{Creatinine} + H_2O \xrightarrow{\text{Creatinine amidohydrolase}} \text{Creatine}$$

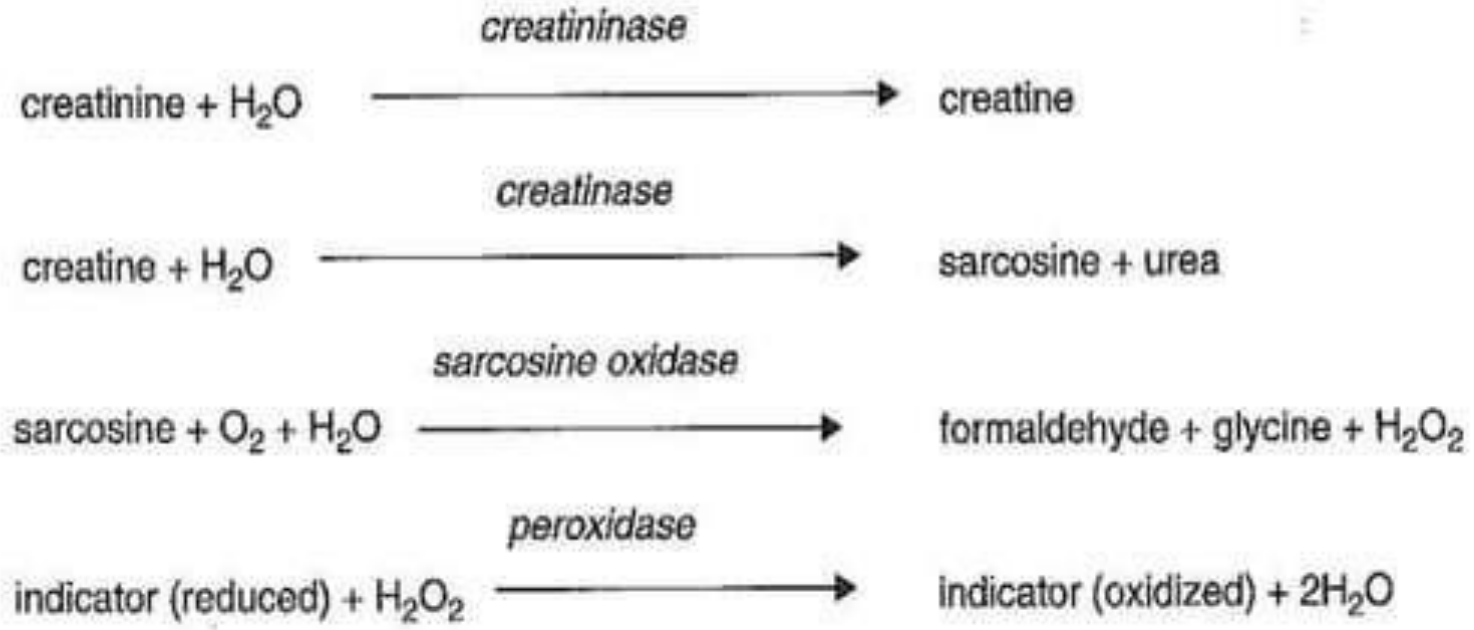
$$\text{Creatine} + \text{ATP} \xrightarrow{\text{Creatinine kinase}} \text{Creatine phosphate} + \text{ADP}$$

$$\text{ADP} + \text{PEP} \xrightarrow{\text{Pyruvate kinase}} \text{ATP} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{Lactate dehydrogenase}} \text{Lactate} + NAD^+$$

Creatininase & Creatinase

- Used in POCT devices using polarographic detection .
- Interference can be removed by using bilirubin oxides or potassium cyanide



CHEMICAL METHOD :

The *Jaffe Reaction* (most commonly used method).

Jaffe reaction is not specific for creatinine

Because many substances produce Jaffe like chromogenic effect including proteins ,glucose, ascorbic acid,& ketone bodies

Non-creatinine chromogenic do not generate interference with urinary creatinine measurement

Principles of the Procedure

Creatinine reacts with picric acid in an alkaline medium to produce a red colored creatinine picrate complex .

OH

Creatinine + Picric acid ⟶ Creatinine-Picrate

The rate of complex formation is measured at 505/571 nm and is directly proportional. to the creatinine concentration

In automated analyzer first reading is recorded during 20 seconds after mixing sample & reagent .(acetoacetate)

2nd reading is noted between 20 & 80 seconds ,b/c creatinine & picrate react slowly.

Window period – 20 to 80 seconds

Reagent 1 -Sodium hydroxide

Reagent 2 - Picric acid

Interference

- Positive interference:
- **Total Protein** at 12.0 mg/dL increases the creatinine result in serum/plasma at 1.71 mg/dL.
- **Glucose** at 332 mg/dL increases the creatinine result in serum/plasma at 1.61 mg/dL
- **Dopamine** at 100 mg/dL increases the creatinine result in serum/plasma at 1.53 mg/dL
- **Negative interference :**
  - **Unconjugated bilirubin** at 15 mg/dL *decreases* the creatinine result in serum/plasma at 1.62 mg/dL
  - **Unconjugated bilirubin** at 20 mg/dL decreases the creatinine result in serum/plasma at 5.32 mg/dL

Creatinine clearance :

Clearance is defined as volume of plasma cleared of that substances in one minutes/s

Expressed in ml/min or ml/sec

Its is directly proportional to body surface area

A 24 hours urine collection is required .

Height & weight of patient is noted

A blood sample is drawn between urine collection

Time = 24×60 = 1140

Urine volume = 1000-2000ml

Serum/plasma creatinine= 1.0mg/dl

Creatinine Clearance corrected

$$\frac{U}{S} \times V \times \frac{1.73}{A}$$

$$\frac{\text{Urine creatinine } U}{\text{Serum creatinine } S} \times \frac{\text{Urine volume (mL) } V}{\text{Collection time (minutes)}} \times \frac{1.73}{\text{Body surface area (m2) } A}$$

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Problems associated with Ccr :

Timed 24 hours urine specimen is burdensome

Incomplete emptying of bladder

Failure to collect entire specimen

Creatinine secretion increase with as GFR dec

Wide inter & intra individual variation

In our lab :

The Atellica CH Crea_2 assay is a modification of the Jaffe method, using rate blanking and intercept correction.

Rate blanking is used to minimize bilirubin interference.

Also, because non-specific serum/plasma protein interactions with this reagent have been found to produce a positive bias of approximately 0.3 mg/dL (26.5 µmol/L), serum/plasma measurements are automatically corrected by subtracting 0.3 mg/dL (26.5 µmol/L) from each result

References :

Chemical pathology for beginners

Tietz 6th edition volume 1 chapter 32 kidney function tests

Thank you .....