

# Estimation of Arginase Activity in liver extract

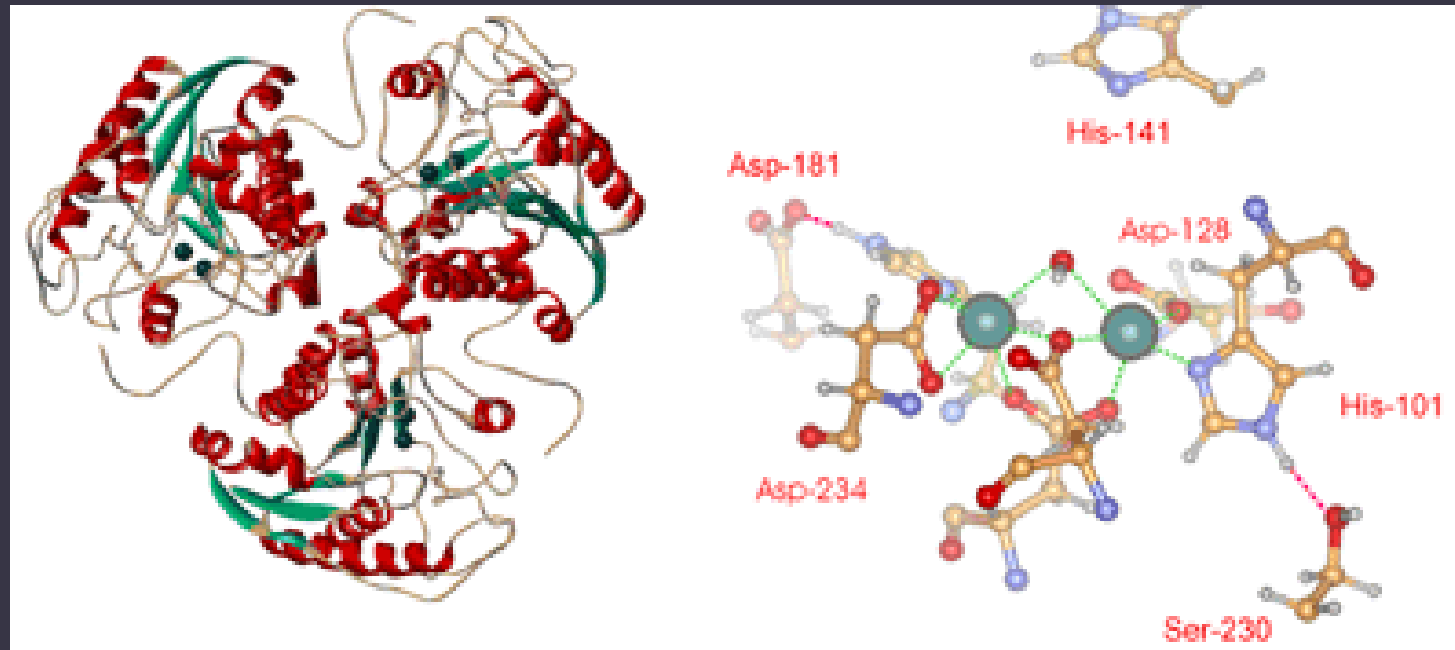
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Arginase is a manganese metalloenzyme that catalyzes the hydrolysis of L-arginine to form L-ornithine and urea. Arginase is the terminal enzyme of the urea cycle among the six enzymes.

The enzyme was found to exist in two forms and has a broad tissue distribution.

1. Arginase I is highly expressed in liver the and is important in ureogenesis., functions in the urea cycle, and is located primarily in the cytoplasm of the hepatic cells.



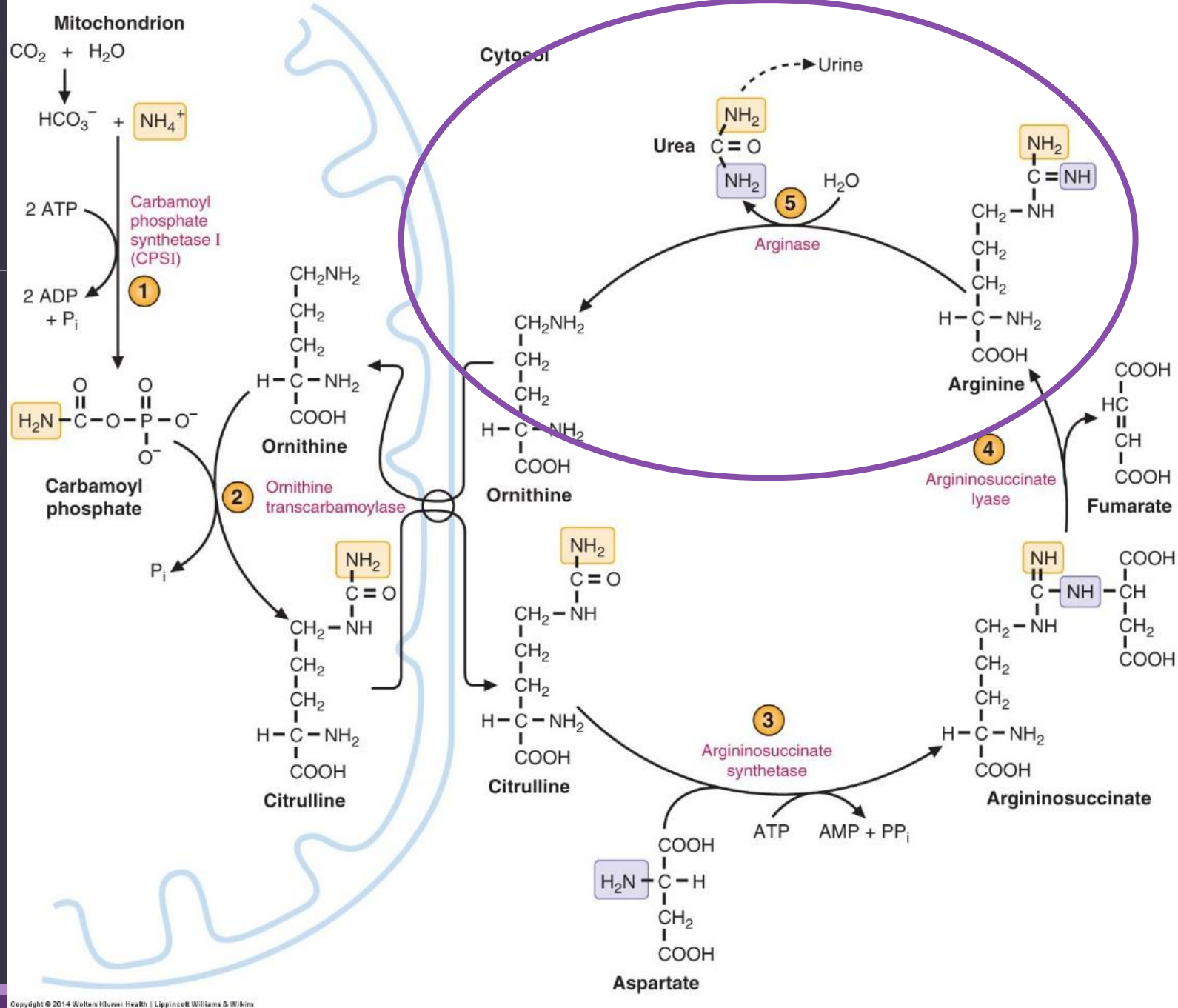
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**Arginase II**, has been implicated in the **regulation** of the arginine/ornithine concentrations in the cell. It is located in mitochondria of several tissues in the body, with most abundance in the kidney and prostate

**Arginase II** is an extra-hepatic All form is thought to be involved in the biosynthesis of polyamines, the amino acids ornithine, proline and glutamate and in the inflammatory process, among others . Recently studies have shown that increased stimulation of arginase expression in animal systems leads to production of polyamines that promote tumor cell proliferation and wound healing .

arginine + H<sub>2</sub>O → ornithine + urea.

Arginase catalyzes the **fifth and final step** in the urea cycle. Specifically, arginase converts L-arginine into L-ornithine and urea



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urea cycle: a series of biochemical reactions in mammals during which the body disposes of harmful ammonia

# Inherited Defects in Urea Cycle Enzymes

Defective enzyme	Metabolites that accumulate in the blood/urine	Comments
Carbamoyl phosphate synthetase I	<b>Ammonia</b>	A very <b>rare</b> disorder, no treatment exists, and the disorder is <b>fatal</b> .
Ornithine transcarbamoylase	<b>Ammonia</b> , orotic acid	An <b>X-linked</b> disorder, <b>most common</b> of the inherited disorders in the urea cycle.
Argininosuccinate synthetase	<b>Ammonia</b> , citrulline	The <b>second most common</b> urea cycle defect, <b>mental retardation can result if not rapidly treated</b> .
Argininosuccinate lyase	<b>Ammonia</b> , argininosuccinate	The NH <sub>3</sub> accumulation is <b>not as severe</b> as the other defects as two nitrogens have been fixed into argininosuccinate, which is now excreted. <b>Arginine is now an essential amino acid</b> .
Arginase	<b>Ammonia</b> , arginine	As argininosuccinate lyase deficiency, the NH <sub>3</sub> accumulation is <b>not as severe</b> as previous defects owing to nitrogens being fixed into arginine, which is excreted.

# What is arginase deficiency?

**other names use for arginase deficiency:**

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ARG1 deficiency , Arginase Deficiency Disease, Argininemia,Hyperargininemia

*Arginase deficiency* is an inherited disorder that causes the amino acid arginine to accumulate gradually in the blood. Ammonia, which is formed when proteins are broken down in the body, is toxic if levels become too high. The nervous system is especially sensitive to the effects of excess ammonia.

**Arginase deficiency usually becomes evident by about the age of 3. The effects of this can include:**

poor growth learning delays ,poor coordination and balance problems,fussiness or illness when fed high protein food

**If untreated, other symptoms can follow:**

muscle weakness ,breathing problems, swelling of the brain ,Coma and death

## Objective:

Estimation of Arginase Activity in liver extract

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### RANGE OF EXPECTED VALUES

SPECIMEN	UREA NITROGEN	UREA
Serum/Plasma	5-23 mg/dL(1.7-8.3 mol/L)	10-50 mg/dL (1.7-8.3 mol/L)

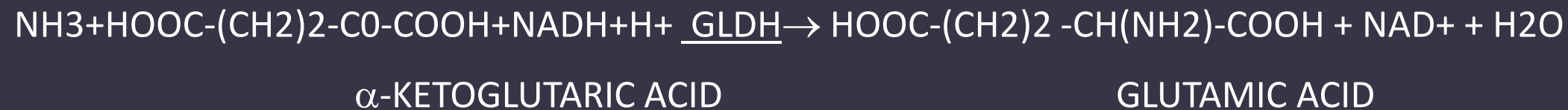
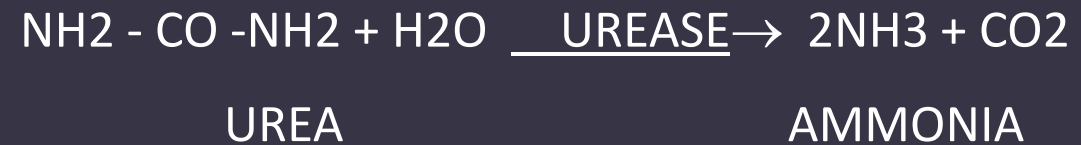
# Principle

Enzyme activity: define as the amount of enzyme that catalyzes the transformation of one micromole of substrate per min. under decreed condition

\*By determine the amount of urea :Urea is hydrolyzed in the presence of urease and water to yield ammonia and carbon dioxide. The ammonia reacts with  $\alpha$ -ketoglutamic acid and reduced nicotinamide adenine dinucleotide (NADH) , then in the presence of glutamate dehydrogenase (GLDH) to yield glutamic acid and nicotinamide adenine dinucleotide (NAD). The rate of oxidation of NADH to NAD is measured at 340 nm over a limited urea concentration range and limited time period, and is proportional to the concentration of urea present.



# Principle



Urea is hydrolyzed in the presence of urease enzyme and water to yield ammonia and carbon dioxide. The ammonia reacts with  $\alpha$ -ketoglutaric acid and reduced nicotinamide adenine dinucleotide (NADH) in the presence of glutamate dehydrogenase (GLDH) to yield glutamic acid and **nicotinamide adenine dinucleotide (NAD)**.

The rate of oxidation of NADH to NAD is measured at 340 nm over a limited urea concentration range and limited time period, and is proportional to the concentration of urea

# Materials

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## Source of enzyme

A sheep liver sample was collected from the local slaughterhouse. A portion of liver tissue was removed within 15 min after sacrificing the animal. It was placed in ice bath and brought to the laboratory as early as possible. The liver sample was immediately used for the isolation and purification of arginase.

## Chemicals:

- 1. Fresh liver
- 2. potassium phosphate Buffer
- 3. ice cold water
- 4. Urea standard
- BUN-ZYME Reagent.
- • **BUN-ZYME Standard solution** 25 mg/dl ( nitrogen = 53.57 mg/dl)

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## Instruments:

- Water bath 37 °C
- Micro pipette
- Homogenizer
- Quartz cuvette
- Stopwatches
- centrifuge

# Method

(Part1): Preparation of the sample ( Extract Arginase from sheep liver): \_\_\_\_\_

1. Homogenize the liver in potassium phosphate Buffer (Equal to 2 times its wet weight)
2. Then the homogenate is centrifuged in cold for 2 min. ,8000rpm.
3. Take the supernatant which contains The Enzyme “Most kept it in cold”

**It is important You must measure the volume of supernatant.**

4. dilute the liver extract 1:5 with ice cold water.(1 ml crud + 4 ml cold water)

# Method

## (Part2): Measuring the arginase Enzyme activity by monitoring the urea

Following the instruction of urea kit

	Standard	test
Reconstituted reagent	1 ml	1 ml
Pre-warm at 37°C for 2 min. then add:		
Standard	0.5 ml	
diluted crude		0.5 ml

- After exactly 30 seconds read and record absorbance A1 against distilled water at 340 nm.
- At exactly 60 seconds after the A1 (read after 30 sec.), read and record the absorbance A2 and determine  $\Delta A$ .
- Urea (mmole/L) =  $(\Delta A \text{ Test} / \Delta A \text{ std.}) \times 8.9$

# Results

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<b>Tube</b>	<b>Standard</b>		<b>test</b>	
<b>Absorbance at 30 seconds</b>				
<b>Absorbance at 60 seconds</b>				
<b>Mean</b>				
<b><math>\Delta A</math> ( use the mean )= A30-A60</b>				

## Calculations:

### How to measure the total Arginase activity in liver extract ( $\mu\text{mole}/\text{min}$ )

- $\mu\text{mole}/\text{min}$  : Total amount of arginine (Urea) in  $\mu\text{mole}$  converted to product per min
- Liver weight =
- Buffer = 2 time of its wet weight
- D.F = 5
- The total volume of supernatant =
- In this experiment you used 1.5 ml from diluted liver extract
- You determine the concentration of urea by urea kit (mmole/L)

# Questions

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What is the EC number of arginase? and in which group of enzymes it belongs to?

From pervious studies find the kinetics of human arginase ; optimum PH , optimum temperature and the km for arginase to arginine.



# References

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<http://www.newbornscreening.info/Parents/aminoacid disorders/argininemia.html>

<http://nutrition.highwire.org/content/134/10/2760S.full>