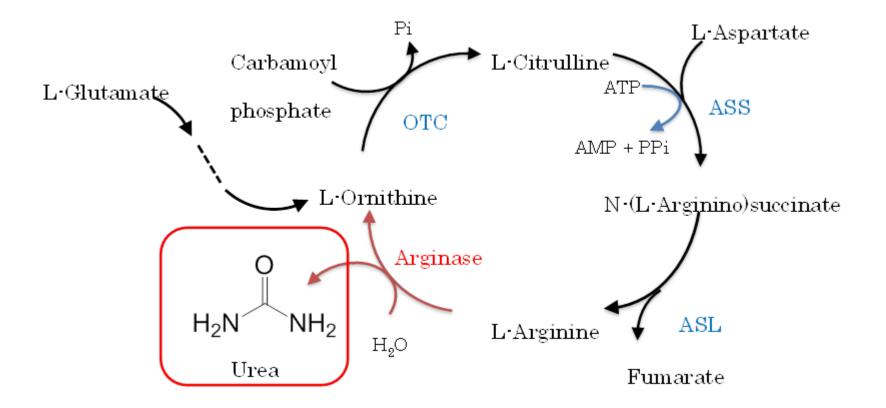
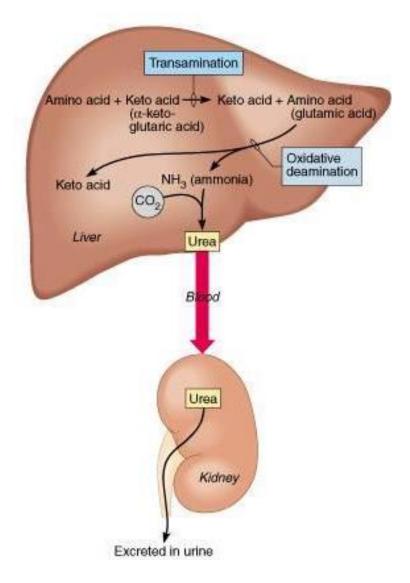
Determination of blood urea



NTRODUCTION:

- Urea is one of the end products of protein metabolism. Some of it is derived from food, and some from the breakdown of tissues.
- It is eliminated from the blood stream by the kidney, and passes out in the urine. In health, blood always contains some urea, the level varying between 14 and 43 mg per 100 ml.

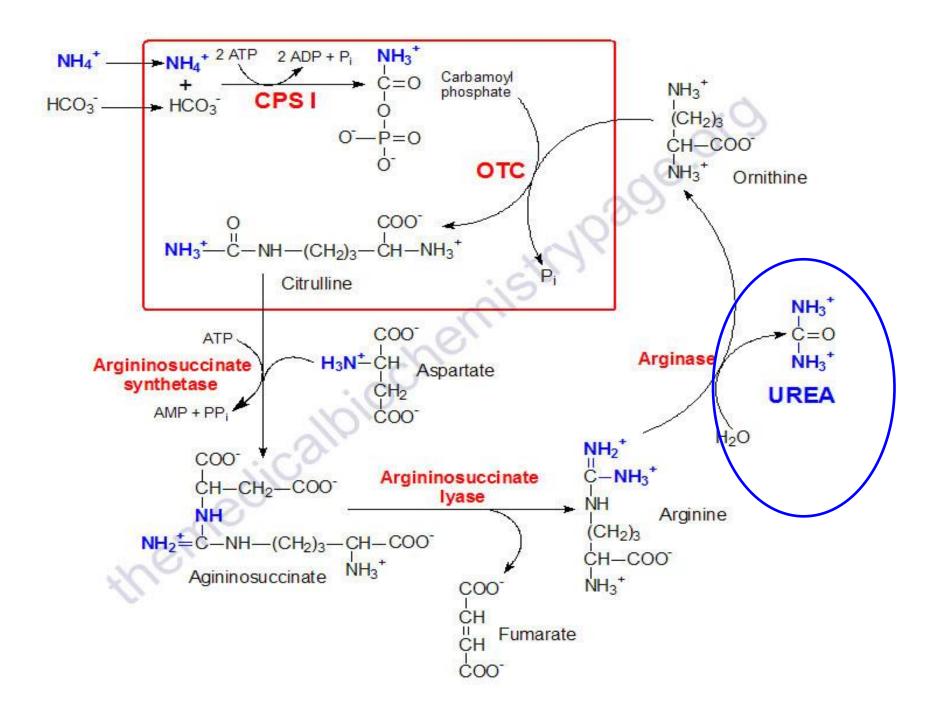


- In the elderly, values slightly higher than these may be present, even without significant renal dysfunction.
- In general, blood urea of over 50 mg per 100 ml is suggestive of impaired renal function; less frequent causes of raised blood urea are diarrhea and vomiting and circulatory failure.
- In childhood and pregnancy, *low* values are often found.

Urea is one of the major end products of protein nitrogen metabolism. It is synthesized in liver from ammonia which is produced by amino acids deamination.

The determination of serum urea nitrogen is an important index of kidney function.

impaired renal function or increased tissue breakdown are associated with increased urea nitrogen levels.



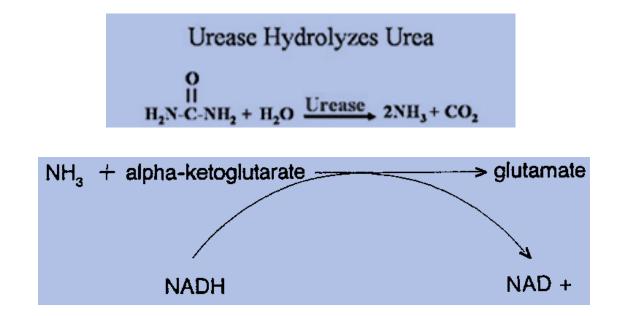
 Urea diffuses very readily through body fluids. For this reason, similar results are obtained if the estimation is carried out on whatever samples are most readily procurable, for example, cerebrospinal fluid, edema, fluid plasma, serum or whole blood.

Normal Values Range

| Specimen | Urea Nitrogen | Urea |
|-----------------------|-------------------|---------------------|
| <u>Serum / Plasma</u> | <u>5-23 mg/dl</u> | <u>10-50 mg/ dl</u> |
| Urine 24 h | 6-19 mg/dl | 20-35 mg/dl |

- PRINCIPLE:

- Urea is hydrolyzed in the presence of urease enzyme and water to yield ammonia and carbon dioxide. The ammonia reacts with αketoglutaric 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.

NH2 - CO -NH2 + H2O UREASE 2NH3 + CO2 UREA AMMONIA

NH3+HOOC-(CH2)2-C0-COOH+NADH+H⁺ GLDH HOOC-(CH2)2-CH(NH2)-COOH+NAD⁺+H2O

 α -KETOGLUTARIC ACID

GLUTAMIC ACID

Material

- BUN-ZYME Reagent: UREASE, GLDH, NADH, α-KETOGLUTARIC ACID, buffers and stabilizers.
- BUN-ZYME Standard solution 25 mg/dl (nitrogen = 53.57 mg/dl)
- BUN-ZYME Serum sample
- Water bathe 37 °C
- Micro pipette
- Quartz cuvett
- Stopwatches



| | Standard | Test | |
|--------------------------------------|----------|-------|--|
| Reconstituted Reagent | 3ml | 3ml | |
| Pre-warm at 37°C for 2 min. and add: | | | |
| Standard | 0.025ml | - | |
| Sample (serum) | - | 0.025 | |

After exactly 30 seconds . read and record absorbance A1 against distilled water at 340 nm. At exactly 60 seconds, read and record the absorbance A2 and determine ΔA

- Calculation:
- ΔA = A1- A2

- Urea= ΔA sample x 53.57 = mg/dl ΔA standard

- Urea nitrogen = ΔA sample x 25 = mg/dl ΔA standard

Thank you