Non-Protein Nitrogenous Compounds

NPN’s

Urea (BUN)

Creatinine

Uric Acid

Ammonia
Non-Protein Nitrogenous Compounds

Urea

- Metabolic product derived from catabolism of proteins
- Proteolysis of proteins yields amino acids
- Deamination of amino acids produce ammonia

\[
\text{RCNH}_2\text{COOH} \rightarrow \text{RCHOCOOH} + \text{NH}_3
\]

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<tr>
<th>Amino acid</th>
<th>Keto acid</th>
<th>Ammonia</th>
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- Ammonia also produced by bacterial degradation of dietary protein in intestinal tract
- Ammonia toxic to CNS
- Converted to less toxic metabolites:
  - urea
  - glutamine

Urea Cycle

- Occurs in liver; enzymes required for urea synthesis found ONLY in liver
- Synthesized urea diffuses out of liver into bloodstream
- Urea filtered through renal glomerulus
- Approximately 40% reabsorbed by passive diffusion in PCT
- Remainder excreted by the kidneys
Glutamine Production

- Second mechanism for removal of NH$_3$
- Occurs in liver, skeletal muscle and brain

BUN (Blood Urea Nitrogen) VS. UREA CONCENTRATION

MW of urea = 60 g/mole
Atomic weight of N = 14 g/mole
2 moles of N per mole of urea = 28 g urea N / mole urea
   = 28 g urea N / 60 g urea
   = 1 g urea N / 2.14 g urea

BUN concentration X 2.14 (60/28) = Urea concentration

Example: BUN = 12 mg/dL
Urea = 12 X 2.14 = 26 mg/dL
BUN (Blood Urea Nitrogen) VS. UREA CONCENTRATION

To convert BUN to mmol/L (International System of Units)

- Convert BUN to urea in mg/dL by multiplying by 2.14
- Multiply by 10 to convert dL to L
- Divide by MW of urea to convert mg/L to mmol/L
  
  MW of urea = 60 g/mole

Example: BUN = 12 mg/dL

  Urea = 12 X 2.14 = 26 mg/dL
  = 26 X 10 = 260 mg/L
  = 260/60 = 4.3 mmol/L

Combining the steps: Urea = 12 X 0.36 = 4.3 mmol/L

Urea

- **AZOTEMIA**: Increased concentration of nitrogenous waste products in blood

- Majority of NPN is urea → azotemia = hyperuremia

Hyperuremia Causes

- PRE-RENAL (NON-RENAL)

- RENAL

- POST-RENAL
Urea

**Pre-Renal Causes of Hyperuremia**
- dehydration (vomiting, excessive sweating, etc)
- reduced blood flow to kidneys
- shock
- congestive heart failure
- severe burns
- high protein intake
- hemorrhage
- tissue necrosis

**Renal Causes of Hyperuremia**
- renal failure
- glomerulonephritis
- renal tubular necrosis
- nephrosclerosis

**Post-Renal Causes of Hyperuremia**
- obstruction due to stones, tumors, enlarged prostate
Non-Protein Nitrogenous Compounds

Urea

Hypoureemia Causes

- severe liver disease
- low protein intake
- pregnancy
- androgens
- growth hormone

Urea: Measurement

Sample: serum or plasma;
analyze within a few hours of collection or store at 4°C to prevent bacterial decomposition of urea

Enzymatic Method

\[
\text{Urea} + \text{H}_2\text{O} \xrightarrow{Urea} (\text{NH}_4)_2\text{CO}_3 \rightarrow 2\text{NH}_4^+ + \text{CO}_3
\]

\[
\text{NH}_4 + \alpha\text{-Ketoglutarate} + \text{NADH} \xrightarrow{GLDH} \text{NAD}^+ + \text{glutamic acid}
\]

Decrease in absorbance at 340 nm proportional to urea conc.

Conductimetric Method

\[
\text{Urea} + \text{H}_2\text{O} \xrightarrow{Urea} (\text{NH}_4)_2\text{CO}_3 \rightarrow 2\text{NH}_4^+ + \text{CO}_3
\]

Measure increase in conductivity as \(\text{NH}_4^+\)}
**Ammonia**

- Ammonia can diffuse across blood-brain barrier – toxic

- Causes of hyperammonemia
  - severe liver disease
  - Reye's Syndrome
  - inherited deficiencies of urea cycle enzymes

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**Inherited Urea Cycle Disorders**

**Citrullinemia**

- Type I defect in argininosuccinic acid synthetase
- Type II defect in production of citrin

**Argininosuccinic Aciduria**

- Defect in argininosuccinic acid lyase
Ammonia: Measurement

Sources of falsely elevated ammonia levels

- Deamination of amino acids in the specimen after sample collected
  - to minimize:
    - collect without trauma
    - use EDTA or heparin anticoagulant
    - place on ice immediately
    - centrifuge and remove from cells within 20 minutes
    - analyze within 3 hours or freeze at -20°C
- Avoid hemolysis (RBC contains 2-3 x as much ammonia as plasma)
- Both patient and phlebotomist should not smoke
- Use chemically clean glassware
- Perform analysis in restricted, ammonia - free area

\[
\text{NH}_4^+ + \alpha\text{-Ketoglutarate} + \text{NADPH} \xrightarrow{\text{GLDH}} \text{NADP}^+ + \text{glutamic acid}
\]

GLDH = glutamate dehydrogenase

Decrease in absorbance at 340 nm proportional to ammonia concentration
Creatine / Creatinine

- Creatine synthesized in liver, kidneys and pancreas from amino acids
- Creatine transported in blood to muscles, brain and other organs

Creatine phosphorylated to phosphocreatine
- High energy source
Creatine / Creatinine

- Creatinine is the anhydride of creatine

Creatinine

- Amount of creatinine produced related to individual's muscle mass - relatively constant production from day to day
- Creatinine filtered by renal glomerulus, no tubular reabsorption, small amount (10-20%) secreted by PCT
- Elevated creatinine levels caused by renal disease
- Creatinine clearance test assesses glomerular filtration rate (GFR)

- Elevations of creatine reflect muscle disease
BUN/ Creatinine Ratio

- BUN / creatinine ratio used to discriminate between causes of hyperuremia
  (Creatinine concentration is not affected by protein catabolism)
- Normal ratio 10:1 to 20:1
- **Ratio <10** (decreased BUN; normal Creatinine)
  severe liver disease, low protein intake
- **Ratio >20 with normal Creatinine**
  pre-renal hyperuremia (high protein catabolism)
- **Ratio >20 with elevated Creatinine**
  post-renal hyperuremia
- **Ratio normal, both BUN and Creatinine elevated**
  glomerular damage (in acute tubular necrosis, relative increase in creatinine can be higher than the BUN resulting in a decreased ratio)

Creatinine: Measurement

**JAFFE REACTION**

Subject to positive bias from glucose, uric acid, glutathione, ascorbic acid, alpha-ketoacids, acetone, acetoacetate, cephalosporins etc.

Sensitive to variations in temperature, pH, time
Creatinine: Measurement

To Improve Specificity of Measurement:

- Add adsorbent containing aluminum silicate to separate creatinine from other non-specific reactants (ie. Lloyd’s reagent)
- Kinetic Jaffe Reaction – measure rate of absorbance change between 20-80 seconds
- Enzymatic – several approaches
Uric Acid

- Uric acid is major catabolic product of the purine nucleosides (adenosine and guanosine)
- Uric acid formed primarily in liver and intestinal mucosa

~70-75% of the synthesized uric acid excreted in the urine freely filtered by renal glomerulus reabsorbed in PCT approximately 50% secreted in tubules back into filtrate some of secreted uric acid reabsorbed in distal tubules

~25% of uric acid secreted into the GI tract
Hyperuricemia

Gout

• Heterogenous group of disorders

• Hyperuricemia caused by over production of uric acid and/or decreased excretion

• Secondary due to drugs and alcohol

• Deposition of urate crystals in tissues → attacks of inflammatory arthritis

• Susceptible to development of renal calculi

Hyperuricemia

Increased Nucleic Acid Turnover

lymphomas
leukemias
polycythemia
cytotoxic drugs
ethanol ingestion
excessive ingestion of purine rich foods

Decreased Excretion of Urate

renal failure
diuretic use
essential hypertension
toxemia of pregnancy
diabetic ketoacidosis
lactic acidosis
Hyperuricemia

Inherited Enzyme Defects

Lesh-Nyhan Syndrome

deficiency of the enzyme hypoxanthine phosphoribosyl transferase (HPRT) enzyme in the purine salvage pathway

Salvage pathways of purines

Hyperuricemia

Inherited Enzyme Defects

Increased activity of phosphoribosyl pyrophosphate (PRPP) synthetase enzyme in the synthesis of purines

Synthesis of purines

Ribose-5-phosphate

ATP

AMP

Phosphoribosylpyrophosphate (PRPP)

Guanine

PRPP-uridyltransferase

5-Phosphoribosylamine

(Many further steps of purine biosynthesis)

Inosine monophosphate (IMP)

Adenosine monophosphate (AMP)

Guanosine monophosphate (GMP)
Hypouricemia

Causes

- severe liver disease
- deficiency of xanthine oxidase (low uric acid accompanied by increased urinary excretion of xanthine)
- defective renal tubular reabsorption

Uric Acid: Measurement

*Folin-Denis*

\[
\text{URIC ACID} + \text{PHOSPHOTUNGSTATE} \rightarrow \text{TUNGSTEN BLUE}
\]

absorbs 650-700 nm

OTHER REDUCING SUBSTANCES FALSELY INCREASE RESULTS

- GLUTATHIONE
- GLUCOSE
- ASCORBIC ACID
- CAFFEINE
- DRUGS
**Uric Acid: Measurement**

**ABSORBANCE**
- HIGH @ 293 nm
- LOW @ 293 nm

**MEASURE:**
- ✓ DECREASE IN ABSORBANCE @ 293 nm
- ✓ COUPLING PEROXIDE FORMED TO A PEROXIDASE REACTION WITH A COLORIMETRIC INDICATOR

\[
\text{H}_2\text{O}_2 + \text{indicator dye } \xrightarrow{\text{peroxidase}} \text{colored compound} + \text{H}_2\text{O} \quad \text{(oxygen acceptor)}
\]