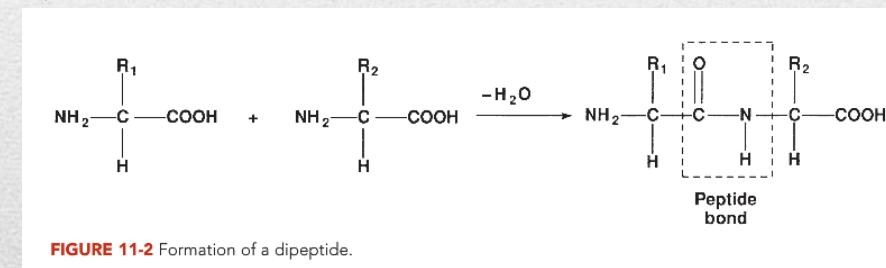


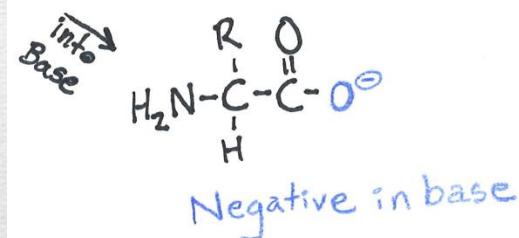
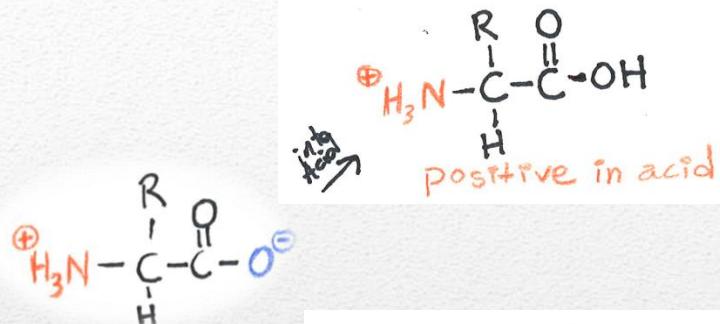
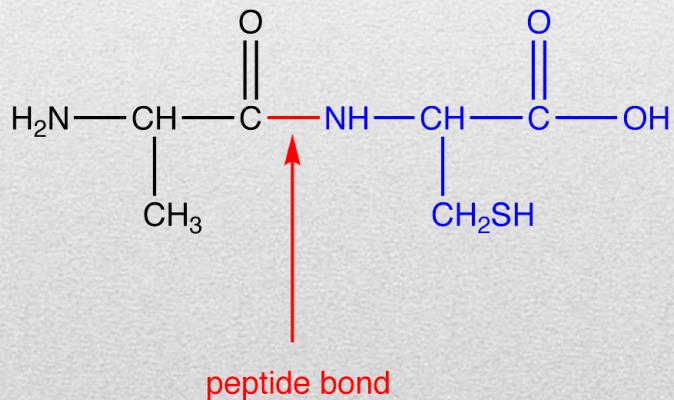
Non-Protein Nitrogens

There's a relation to proteins, but they aren't proteins it's sorta a downstream thing.... You'll see what I mean

- Amino Acids: The building block of proteins
 - Peptide bonds with N and C-terminal ends
 - Cysteine is special: Disulfide bonds
 - Some AA.s are important precursors
 - Tyrosine: Thyroid Hormone, epinephrine, dopamine
 - Tryptophan: serotonin, melatonin



- Dipolar molecule
- Peptide bonds join
 - H₂O taken out



Amino Acids

- Inherited enzyme deficiency
 - More than 100 distinct diseases identified
 - Buildup of amino acids/by-products leads to toxic effects
 - Deficiency of products also an issue
 - Most babies are screened at birth for deficiency
 - Treatment? Avoid amino acids



Aminoacidopathies

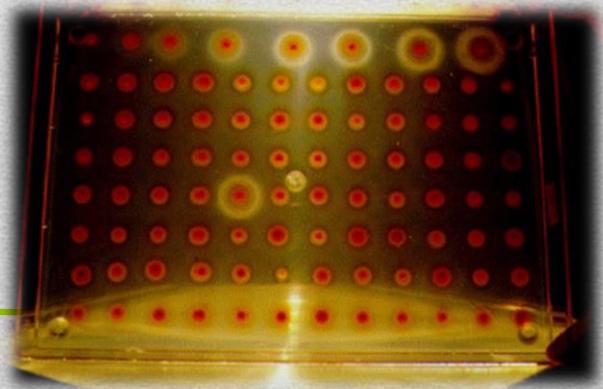
- PKU Phenylketonuria
 - PKU “Classic” is PAH deficiency
 - Brain damaged by buildup
 - DD, microcephaly
 - Urine has musty odor
 - Patient must avoid high protein foods
 - Meat, fish, poultry, eggs, dairy
 - New drugs can increase PAH activity
 - Only works if there is SOME PAH



Aminoacidopathies

- Guthrie Test
 - Petri dish with β -2-thienylalanine
 - *B. subtilis* unable to synthesize phenylalanine in this agar
 - If the patient sample has excess phenylalanine it will grow
 - Modified Guthrie tests can be done for other aminoacidopathies
- Fluorescence test
- Confirmation by HPLC (reference method)

PKU Tests



- Type I
 - Severe, fumarylacetoacetate hydrolase deficient
 - Failure to thrive, diarrhea, vomiting, jaundice, “cabbage odor,” distended abdomen, swelling legs and bleeding
- Type II
 - Tyrosine aminotransferase deficient
 - $\frac{1}{2}$ DD, tearing and photophobia, painful lesions on soles and palms
- Type III
 - Very rare 4-hydroxyphenylpyruvate dioxygenase def.
 - Mild DD, seizures, periodic ataxia

Tyrosinemia

- Homogentisate oxidase
 - Tyrosine and phenylalanine metabolism
 - Urine turns dark brown on mixing with air
 - Homogentistic acid oxidizes to turn black/brown
 - Ochronosis: arthritis-like symptoms, dark spots on sclera
- When ferric chloride added to urine it turns urine black
- High dose vitamin C decreases buildup

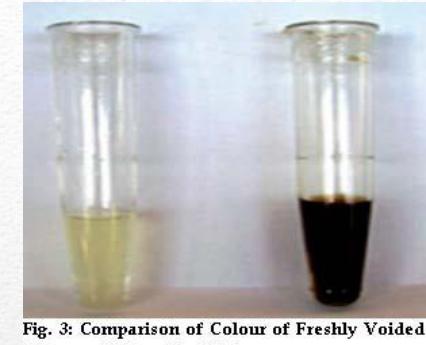


Fig. 3: Comparison of Colour of Freshly Voided Urine and Urine after 24 Hours

Alkaptonuria



- Maple Syrup Urine Disease
 - Branched-chain amino acid buildup
 - Leucine, isoleucine, valine
 - Burnt sugar odor
 - Within weeks of birth lethargy, vomiting, loss of appetite, failure to thrive
 - Then CNS: rigidity, stupor, respiratory irregularity
 - Severe DD, seizures, acidosis, hypoglycemia and death if not treated
- Tested for using modified Guthrie test and fluorescence
- MS/MS can be used, and prenatal amniocentesis for enzyme

MSUD



- Prevents metabolism of leucine
 - Isovaleric acid builds up and smells like sweaty feet
 - Mild → Life threatening
- MS/MS for testing
- Glycine and carnitine can be given to help excrete isovaleric acid

Isovaleric Acidemia

- Cystathione β -synthase deficiency for methionine
 - Methionine builds up in addition to homocystine
- Osteoporosis, dislocated lenses and DD
 - Eventually gets much worse
- Treatment: Restrict methionine, high dose B₆
- Tested for using modified Guthrie test and HPLC or LC-MS/MS
- Patients with atherosclerosis have high homocysteine
 - Also B₁₂ deficient do too

Homocystinuria

- Amino Acid Transport is defective
 - Kidney stones of cysteine crystals form
 - Hematuria, pain, UTIs
- Treatment: High fluid intake (4L/day)
 - Dilute the cystine so it cannot form crystals
 - If that doesn't work, penicillamine forms soluble complexes
 - Nephrolithotripsy
- Testing: Cyanide nitroprusside produces red-purple with the SH groups

Cystinuria

- Fasting samples (6-8 hours)
- Heparinized plasma taken of cells ASAP
 - Hemolysis is unacceptable
- Deproteinization should be done within 30 minutes and sample tested or frozen.
- For screening TLC is common method

Amino Acid Testing

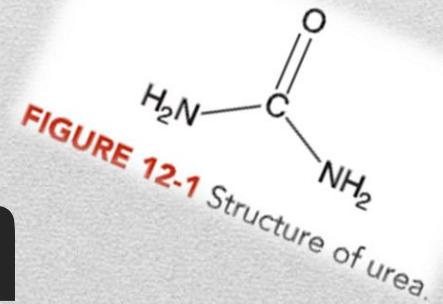
- Nitrogen is the “quintessential” molecule of proteins
 - Historically proteins were precipitated before assaying total nitrogen content
 - Most of the nitrogen is coming from catabolism of nucleic acids and proteins
 - Now we analyze the specific compounds learning certain things from different assays



Non-Protein Nitrates

- Urea
 - Accounts for the largest portion of NPN (45-50%)
 - Product of protein catabolism
 - NH₂ amino groups form ammonia, which is detoxified to urea
 - Most urea is filtered by the glomerulus and urinated out
 - Some is reabsorbed in the tubules, how much changes
 - As such, it can be used to assess renal function and nitrogen balance

Blood Urea Nitrogen

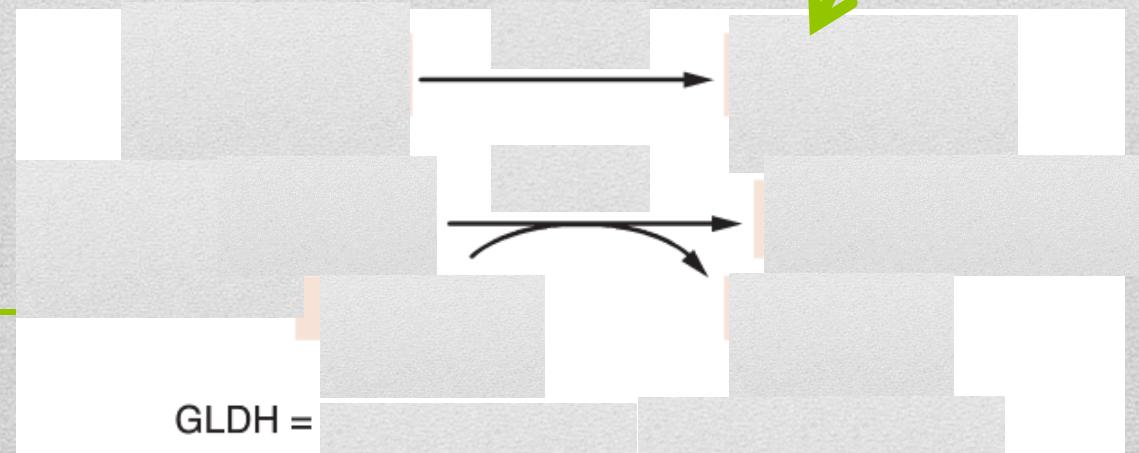


- Urea originally assayed in terms of the nitrogen content
 - We like traditions! So we still do this
 - We convert from BUN to Urea by multiplying by 2.14
 - To convert BUN to mmol/L urea multiply by 0.36
- Testing:
 - Most methods are enzymatic utilizing urease
 - Reference Range: 6-20 mg/dL
 - Citrate and fluoride inhibit urease
 - Ammonium heparin obviously can't be used
 - Urine should be fresh, why?

Causes change in pH, conductivity that's measurable



BUN

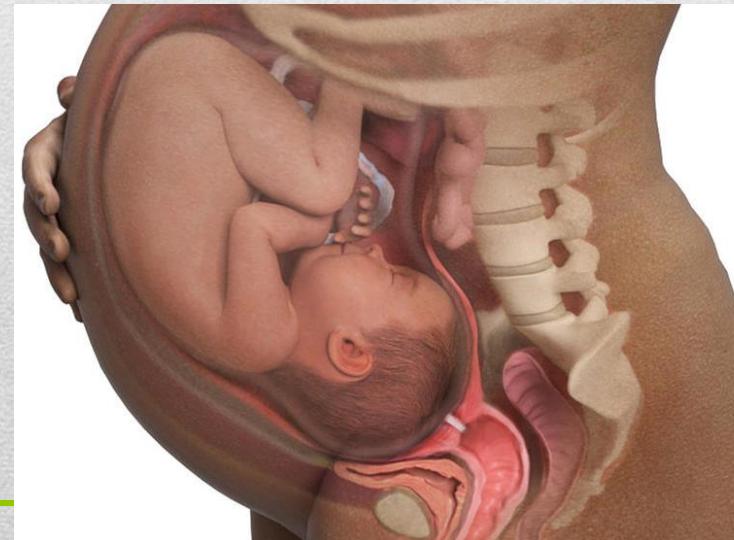


- High BUN is azotemia, very high is uremia
 - Azotemia comes in prerenal, renal, and post renal flavors!
- Prerenal: Reduced blood flow leads to decreased filtering of urea
 - Ex:?
- Renal: Decreased renal function leads to urea building up
 - Ex: acute/chronic renal failure, AGN, tubular necrosis etc...
- Postrenal: Decreased urine flow after the kidney backs it up
 - Ex: Kidney stones, tumor of bladder or prostate, VERY bad UTI

BUN Pathologies

- Decreased BUN?
 - Decreased protein turnover
 - Nutritional deficit
 - Liver disease
 - Getting used up to make proteins for parasitic infestations
- BUN:Creatinine ratio differentiates the azotemia

BUN Pathologies



- Waste product from muscles
 - Creatine produced by liver, phosphorylated in muscles and used as disposable fuel
- Cleared by kidneys
 - Inversely related to GFR
 - Small amounts are secreted and reabsorbed
- Concentration is function of muscle mass, renal function, creatinine turnover, age, gender, race

Creatinine



- A good measuring stick, but not great
 - Not sensitive enough for mild diseases
 - The ideal substance is totally filtered by glomerulus and not reabsorbed at all
- Creatinine Clearance is our tool
 - $$\frac{\text{Urine Creat} \times \text{Urine Volume}}{\text{Plasma Creatinine} \times \text{Time (minutes)}} = \text{mL/min}$$
 - This overestimates GFR because of reabsorption and secretion
 - BSA correction (1.73/Area)

Creatinine and GFR

- Jaffe Method
 - Alkaline picrate + creatinine → red-orange chromagen
 - VERY non-specific
 - Glucose, ketones, vitamin C, pyruvate all interfere
 - Bilirubin and hemoglobin cause negative bias

Creatinine Analysis

TABLE 12-6 SUMMARY OF ANALYTIC METHODS—CREATININE

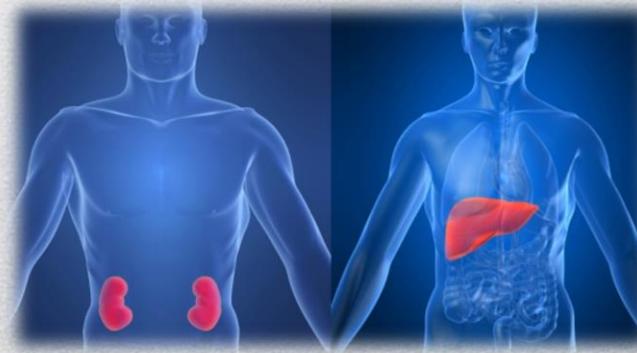
CHEMICAL METHODS BASED ON JAFFE REACTION		
Jaffe reaction	In alkaline solution, creatinine + picrate → red-orange complex	
Jaffe-kinetic	Jaffe reaction performed directly on sample; detection of color formation timed to avoid interference of non-creatinine chromogens	Positive bias from α -keto acids and cephalosporins; requires automated equipment for precision
Jaffe with adsorbent	Creatinine in protein-free filtrate adsorbed onto Fuller's earth (aluminum magnesium silicate), then reacted with alkaline picrate to form colored complex	Adsorbent improves specificity; previously considered reference method
Jaffe without adsorbent	Creatinine in protein-free filtrate reacts with alkaline picrate to form colored complex	Positive bias from ascorbic acid, glucose, glutathione, α -keto acids, uric acid, and cephalosporins
ENZYMATIC METHODS		
Creatininase- H_2O_2	In a series of enzymatically catalyzed reactions, creatinine is hydrolyzed to creatine, which is converted to sarcosine and urea. Sarcosine is oxidized to glycine, CH_2O , and H_2O_2 . Peroxidase-catalyzed oxidation of a colorless substrate produces a colored product + H_2O	Adapted for use as dry slide method; potential to replace Jaffe; no interference from acetoacetate or cephalosporins; some positive bias due to lidocaine
Creatininase-CK	In a series of reactions catalyzed by the enzymes creatininase, creatine kinase, pyruvate kinase, and lactate dehydrogenase, NAD^+ is produced and measured as a decrease in absorbance	Lacks sensitivity; not used widely
OTHER METHODS		
Isotope dilution mass spectrometry	Detection of characteristic fragments following ionization; quantification using isotopically labeled compound	Highly specific; accepted reference method

- Normal ratio 10-20:1
 - Pre-renal conditions elevate the BUN (ratio goes up)
 - Creatinine remains normal
 - Renal conditions elevate them a lot in parallel (ratio ~ same)
 - Post-renal conditions elevate BUN more than Creat (ratio goes up)
 - But creatinine IS raised

	BUN	Creat	Ratio
Pre-Renal	↑↑	Normal	↑
Renal	↑↑	↑↑	Normal
Post-Renal	↑↑	↑	↑

BUN:Creatinine Ratio

- Pre-renal: CHF, hemorrhage, dehydration, protein catabolism, high-protein diet
- Renal: Renal failure, AGN, tubular necrosis
- Post-renal: Obstruction
- Low ratio???
 - Decreased protein intake, severe vomiting/diarrhea, liver disease, pregnancy



BUN:Creatinine Ratio

- Uric acid is not a measure of kidney function
 - Purine catabolism product
 - Insoluble in plasma existing as urate
 - Only will precipitate once plasma saturated
- Most mammals are better at this and metabolize it to allantoin
- When does catabolism go up?
 - High cell turnover
 - Chemo, proliferative disorders, PCV
 - Causes gout and stones

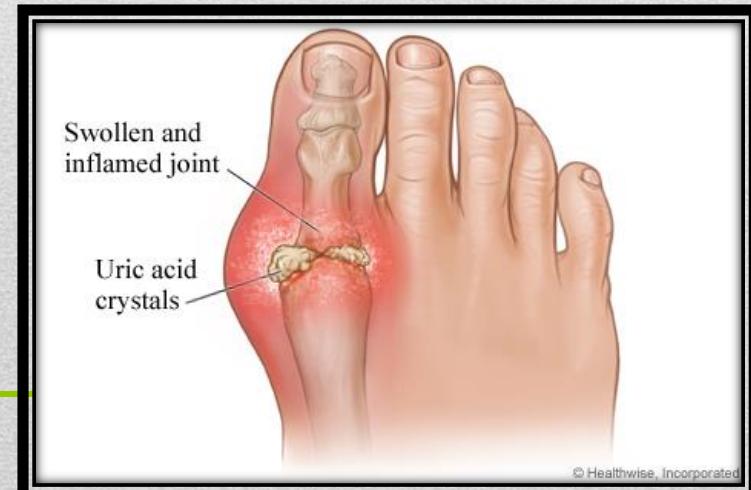
Uric Acid

**And now
for something
completely different...**



- Caraway Method: oxidation of uric acid in PFF
 - Phosphotungstate in alkaline sol'n to tungsten blue
 - Not very specific
- Enzymatic Method: Utilize uricase → Allantoin + H_2O_2
 - Absorbance at 293 nm
 - Hydrogen peroxide and indicator dyes change absorbance

Uric Acid Assay

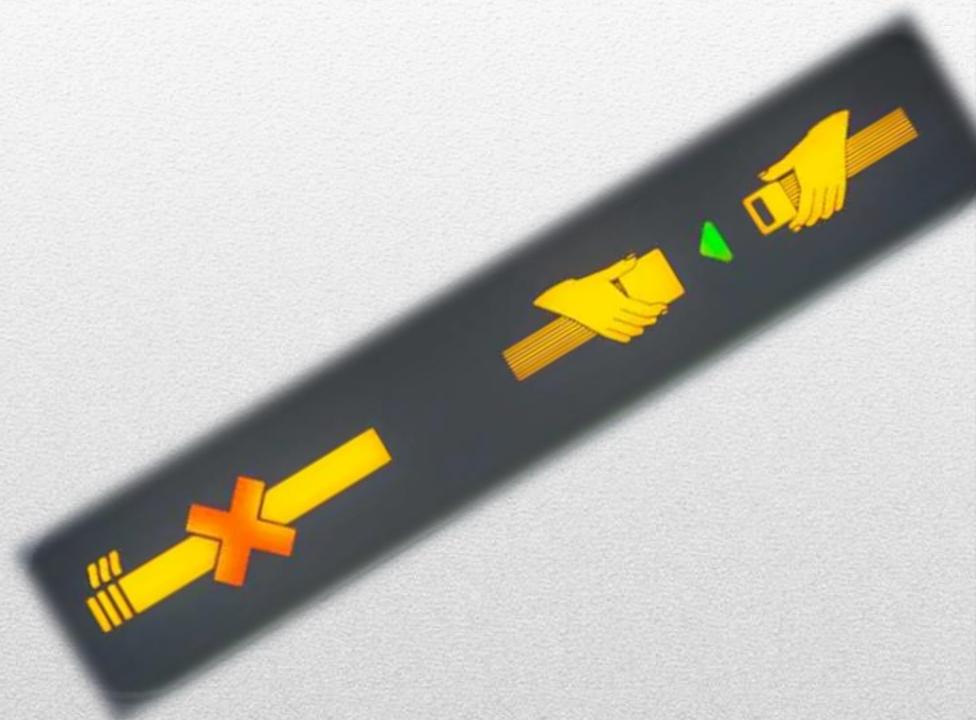


- Deamination waste product
 - Most comes from intestines due to bacteria
 - Turned into urea by liver
 - Elevated in liver failure, Reye's syndrome, urea cycle deficiency
- Hepatic encephalopathy
 - Ammonia can send brain into coma
- Measurement Modalities
 - Separate ammonia out
 - Directly Measure the ammonia

Ammonia



- Allow it to diffuse and change pH
- Enzymatically using GLDH
 - Decrease @ 340 nm
- Bromophenol Blue
- Very delicate specimen
 - Must be kept on ice
 - No hemolysis
 - Smoking is bad, 'mkay?



Ammonia Measurement