GBS 724

The Chemistry of the metabolome

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What is a metabolite?

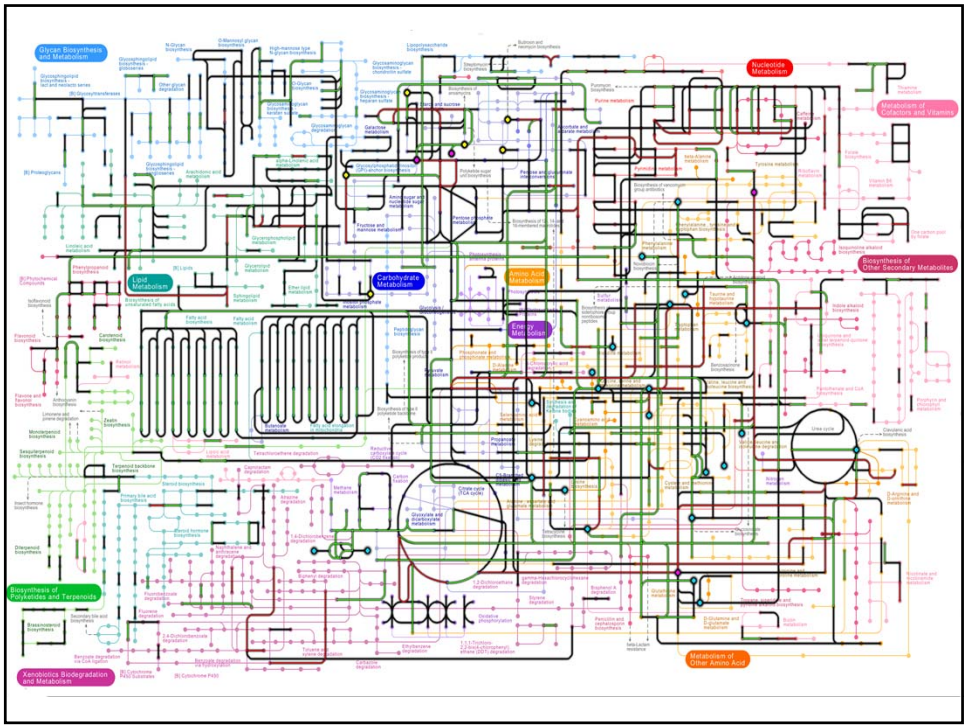
- **In the context of metabolomics, it is *any compound of any origin that has a molecular weight <1,500 Da that is detected in the biological system being studied***
- **This is an arbitary definition**

The metabolome is more than what's in textbooks

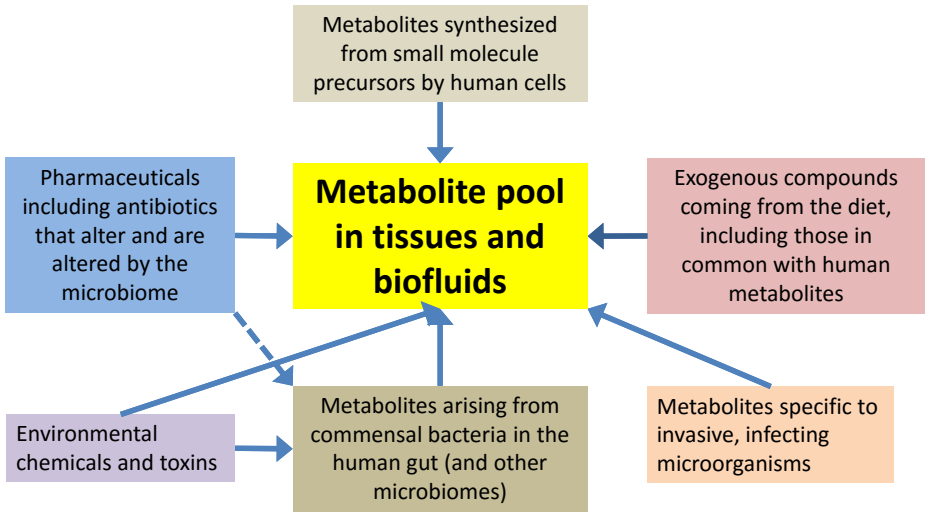
Metabolites synthesized from small molecule precursors by human cells

↓

Metabolite pool in tissues and biofluids



The metabolome is more than what's in textbooks



Overview of metabolite chemistry

- **Metabolites encompass an enormous range of chemistries**
  - **Gaseous**
    - **H_2, H_2S**
  - **Volatile**
    - **Butyric acid, acetone, skatole**
  - **Hydrophilic**
    - **Glucose**
  - **Charged-positive/negative**
    - **Amino acids, nucleotides, organic acids, amines**
  - **Hydrophobic**
    - **Lipids, steroids, hydrocarbons**

Gases and volatiles

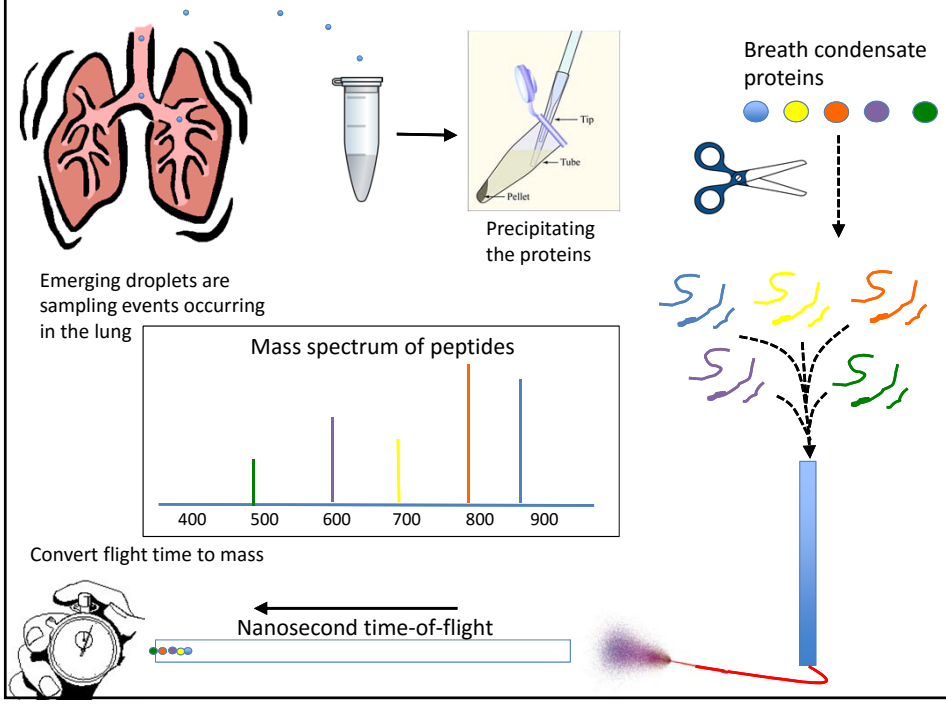
- In breath
  - H_2 from reductive anerobic bacteria
    - Lactose-intolerant
    - Measure of gut transit (typically 4-6 hours)
  - CO_2
    - From all carbon-containing substrates
    - From specific ^{13}C-labelled substrates
  - Acetone (in diabetics)
  - Trimethylamine
    - From fish or FMO3-deficient subjects

Gases and volatiles

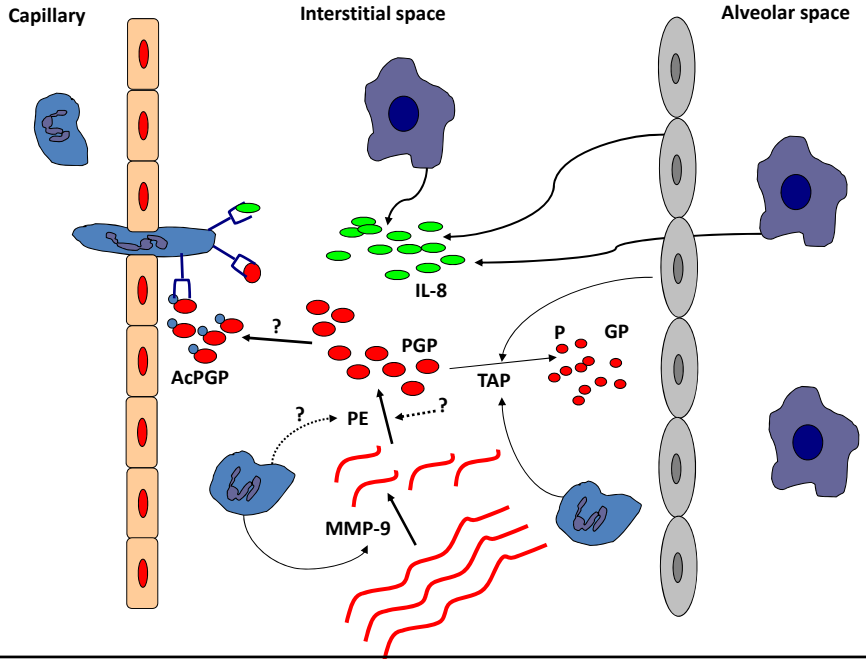
- Sweat gland
  - Sweaty socks syndrome
    - Isovaleric acid (isoleucine metabolism)
- Flatulance
  - Mostly gases (H_2, CO_2 and H_2S), but with volatiles produced by colonic bacteria (skatole)

Breath condensates

- Not strictly consisting of volatiles
- A mist or spray created by the frothing of the fluids inside the lung
  - Condensable using a dry-ice cooled trap
  - Several ml of condensate can be easily collected



A homeostatic circuit controlling neutrophil recruitment



PGP is a common peptide in human collagen

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GPRGPAGPPGRDGIPGQPGLPGP**PGPPGPPGP**PGLGGNFAPQLSYGYDEKSTGGISV**PGP**
MGPSGPRGL**PGP**PGA**PGP**QGFQGPPGEPGEPGASGPMGPRGP**PGP**PGKNGDDGEAGKPGR
PGERGP**PGP**QGARGLPGTAGLPGMKGHRGFSGLDGAKGDAGPAGPKGEPGSPGENGAPGQ
MGPRGLPGERGRPGA**PGP**AGARGNDGATGAAGP**PGP**TGPAGPPGFPGAVGAKGEAGPQGP
RGSEGPQGVRGE**PGPPGP**AGAAGPAGNPGADGQPGAKGANGAPGIAGAPGFPGARGPSGP
QGPGGP**PGP**KGNSGEPGAPGSKGDTGAKGE**PGP**VGVQGP**PGP**AGEEGKRGARGE**PGP**TGL
PGPPGERGGPGSRGFPGADGVAGPKGPAGERGS**PGP**AGPKGSPGEAGRPGEAGLPGAKGL
TGSPGS**PGP**DGKTGP**PGP**AGQDGR**PGPPGP**PGARGQAGVMGF**PGP**KGAAGEPGKAGERGV
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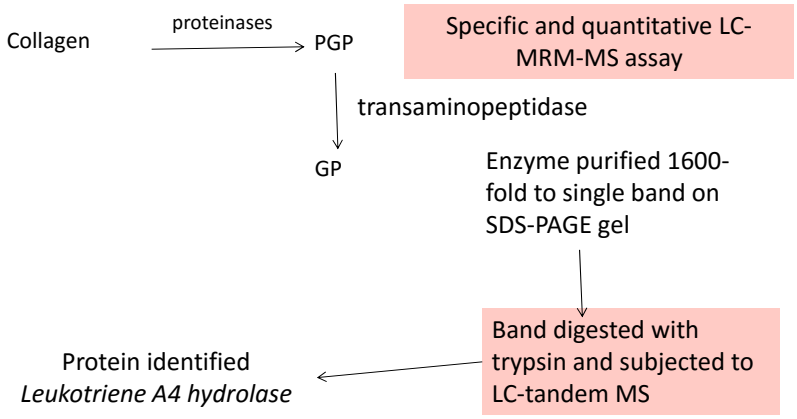
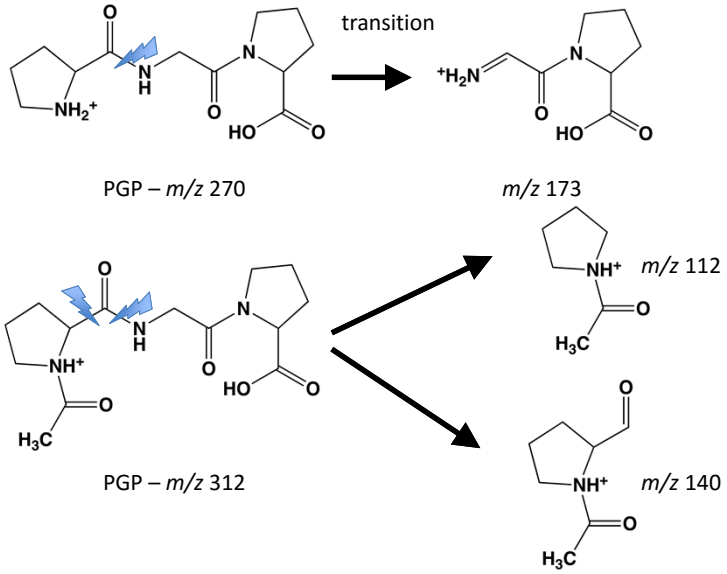
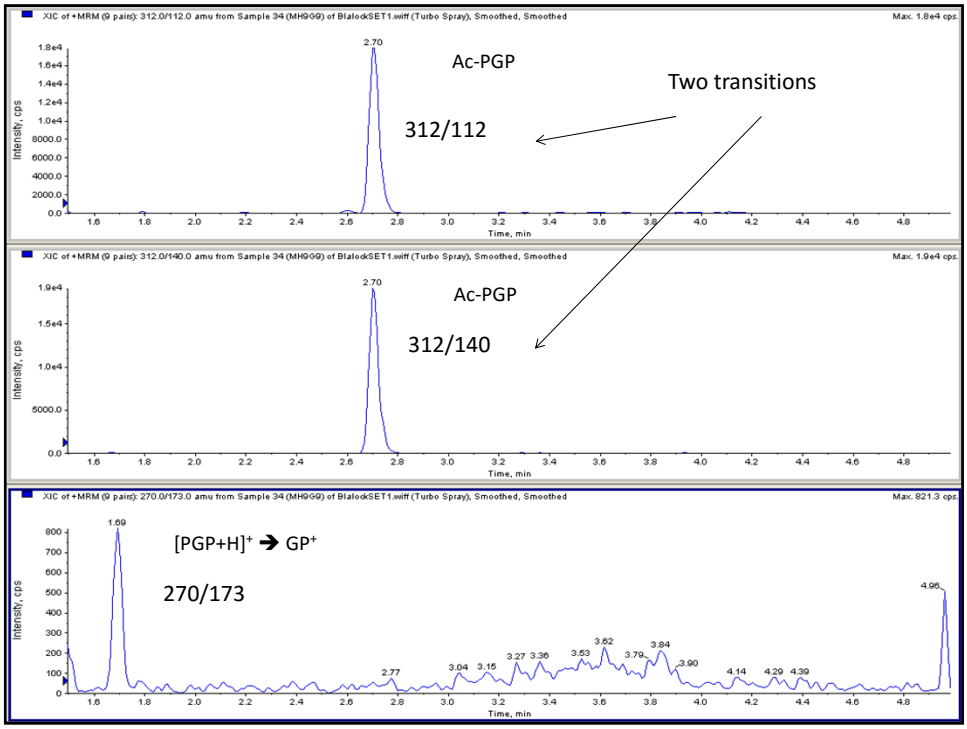
Mass spec contribution to PGP story



(Robert Snelgrove et al. Science, 2010)

Measuring PGP and acetyl-PGP





Metabolopeptidomics or peptidometabolomics

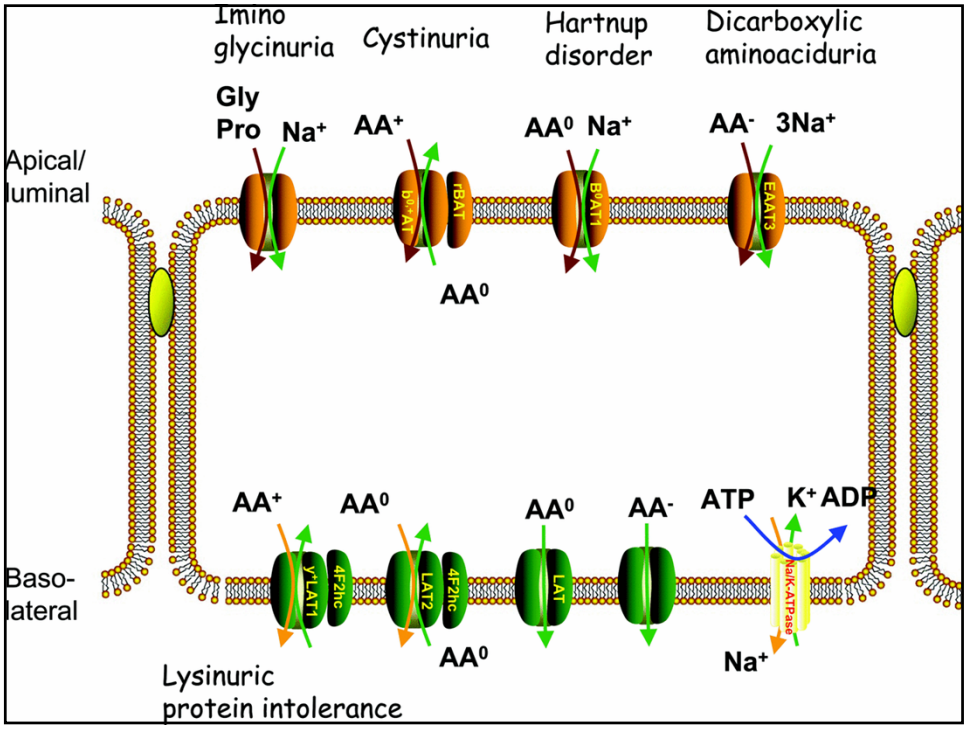
- **Are peptides metabolites?**
- **Are the tripeptides real?**
  Or is their mass simply coincident with the empirical formula of another metabolite?

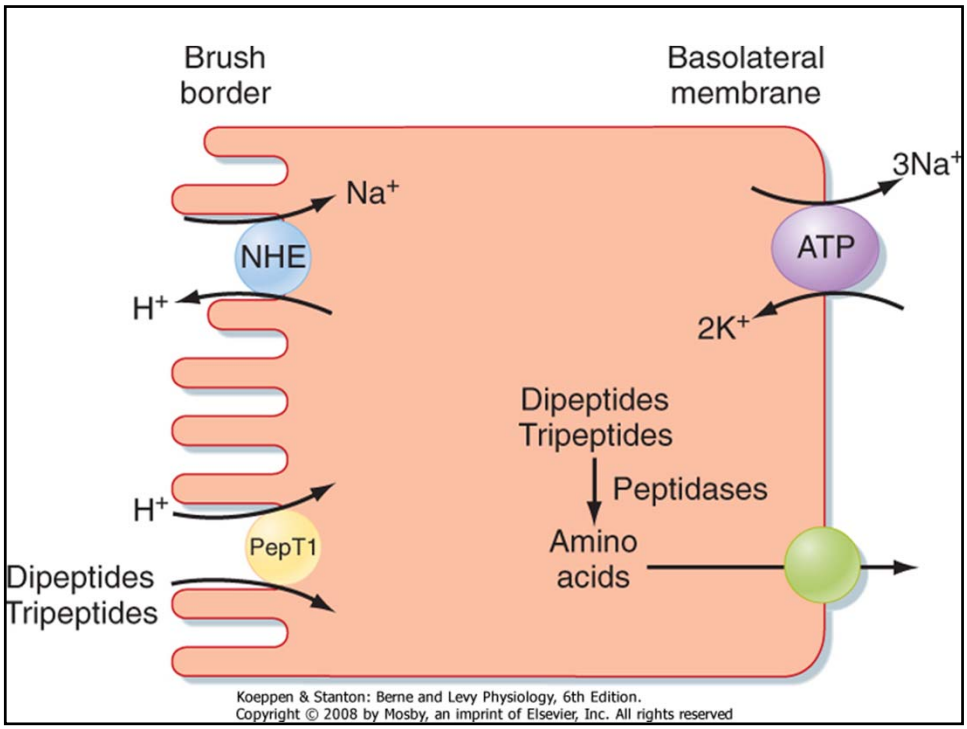
Considering the case for tripeptides

- Examine the basic physiology and pharmacology
- Are there examples of bioactive tri-peptides?
- What about other oligopeptides?
- Where would they come from?
- Why does METLIN seem to always have tri- and not other oligopeptides?

Basic Pharmacology of amino acids

- There are many barriers to compounds within the body
  - Gut-systemic circulation (oral xenobiotics)
  - Blood-brain
- Passage across membranes governed by
  - Membrane solubility/diffusion
  - Facilitated transport
  - Active transport


Imino glycinuria
Cystinuria
Hartnup disorder
Dicarboxylic aminoaciduria
Gly
Pro
Na+
AA+
AA0
Na+
AA-
3Na+
Apical/ luminal
bo,+AT
rBAT
B0AT1
EAAT3
AA0
AA+
AA0
AA0
AA-
ATP
K+
ADP
Baso- lateral
y+LAT1
4F2hc
LAT2
4F2hc
LAT
Na/K-ATPase
AA0
Na+
Lysinuric protein intolerance


Brush border
Basolateral membrane
Na+
NHE
H+
3Na+
ATP
2K+
Dipeptides
Tripeptides
Peptidases
H+
PepT1
Amino acids
Dipeptides
Tripeptides
Koeppen & Stanton: Berne and Levy Physiology, 6th Edition.
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Tripeptides could come from foods, but are hydrolyzed by peptidases in the enterocyte to amino acids

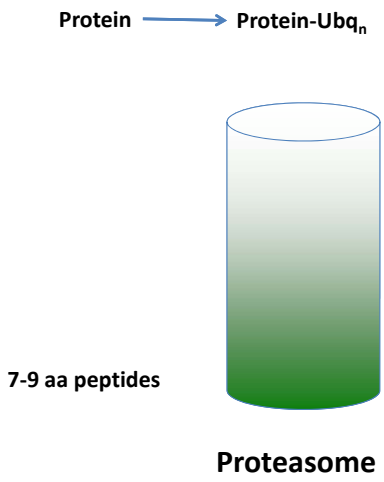
Deficiencies in the peptidases could lead to food and bacterial peptides entering the systemic circulation

Can tripeptides have biological activity?

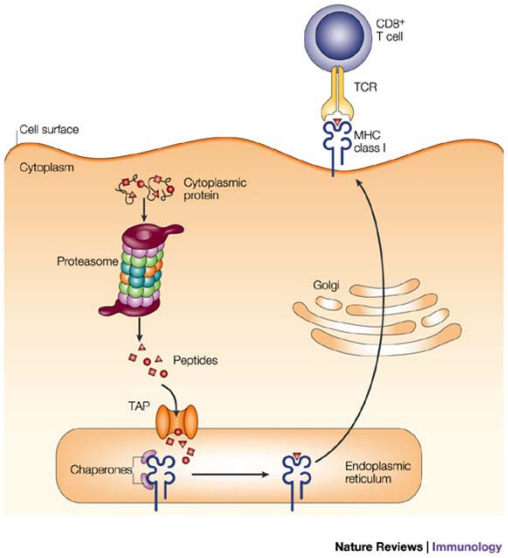
- **For toxicologists, there is one very familiar tripeptide without whom, I would not be giving this talk, or you to listen to it**.
- **Glutathione (GSH) – glutamyl-cysteinyl-glycine**
  - GSH reacts with free radicals to generate GSH conjugates and therefore protects many organs
- **It is synthesized from small molecule precursors**
  - **However, it is a true metabolite, i.e., it is made from smaller precursors without the direct aid of ribosomes**

Are there other sources of short peptides?

- Proteins undergo degradation in the proteasome caused by targeted ubiquitination
  - The digested products are peptides (escapees?)
- Lysosomes
- Autophagosome
- Neutrophil attack
- Other proteases (in renal tubules?)



Antigen processing to peptides by the proteasome and attachment to MHC Class 1 molecules



Other volatiles

- Short chain, unsubstituted fatty acids
  - Formic, acetic, propionic, butyric acids
- Will evaporate in the acidic form
  - Formic acid, b.p. 101°C
  - Acetic acid, b.p. 118°C
  - Propionic acid, b.p. 141°C
  - Butyric acid, b.p. 163.8°C
  - Isobutyric acid, b.p. 155°C
- Convert to ammonium salts before evaporating

Hydrophilic metabolites

- The most extreme hydrophilic metabolites without charged groups are the polyols:
  - Monosaccharides
    - Glucose
    - Fructose
  - Disaccharides
    - Lactose
    - Maltose
  - Oligosaccharides

Organic acids

- Besides the short chain fatty acids mentioned earlier, there are many organic acids representing important cellular pathways
  - Glycolytic intermediates
    - G1P, G6P, F6P, F1,6DP, GAP, DHAP, GP, PEP, Lact
  - Krebs cycle
    - Cit ,cis-Acon, IsoC, aKG, Succ, Fum, Malate, oxaloactate and those resulting from pathway defects
  - Nucleotides
    - ATP, ADP, AMP, GTP, etc.

How could we isolate organic acids?

- Organic acids at neutral pH are negatively charged
- They will bind to anion exchange resins in say the formate form

AG-1

- Can be eluted with ammonium formate or ammonium acetate (mass spec compatible)

Amino acids

- All the α-amino acids found in proteins and their precursors and metabolites
  - Mostly L-isomers, but there are D-isomers in nature
- Tryptophan is oxidized to kynurenine and is a precursor to NAD(H) and NADP(H), serotonin melatonin and niacin
- β-alanine is formed from uracil
  - 5-fluorouracil (anticancer drug) is converted to 2-fluoro-β-alanine which is in turn converted to bile acid conjugates

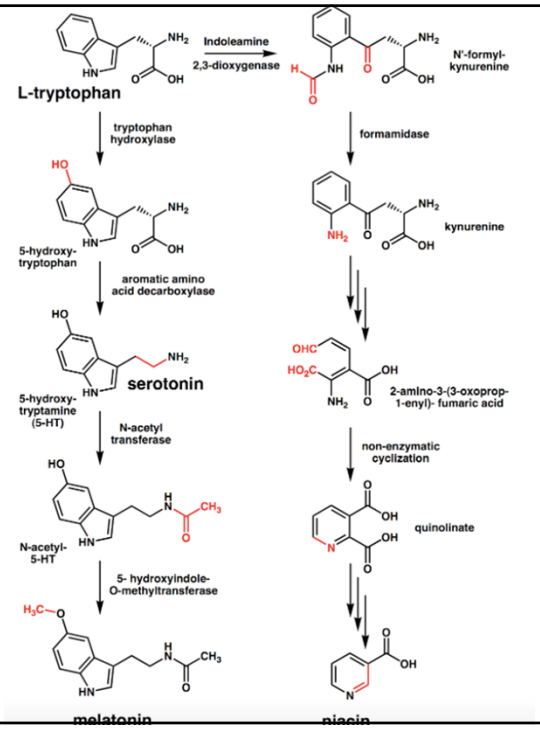
How could we isolate amino acids?

- Amino acids at neutral pH are positively charged
- They will bind to cation exchange resins in the H^+ form

AG-50

- Can be eluted with ammonium hydroxide (mass spec compatible)

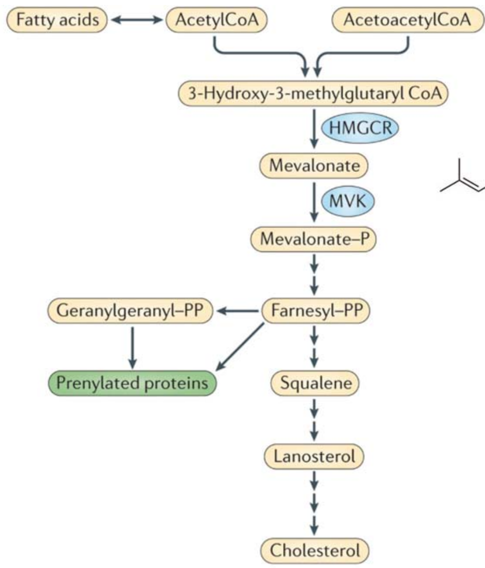
Tryptophan metabolism

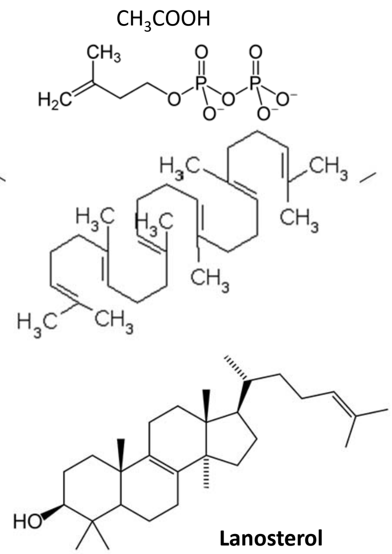


Hydrophobic metabolites

- These include sterols, steroid hormones, terpenoids, bile acids, vitamins A, D, E and K, and a vast array of lipids

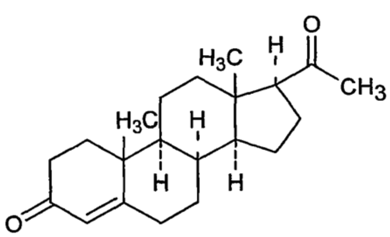
Isoprenoids and sterols



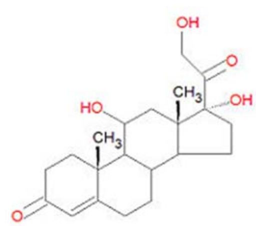


Lanosterol

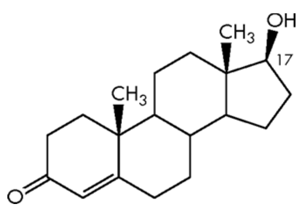
Steroids



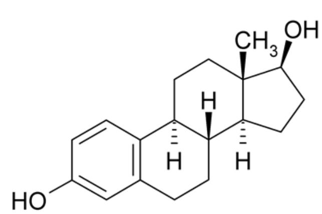
Progesterone



Cortisol

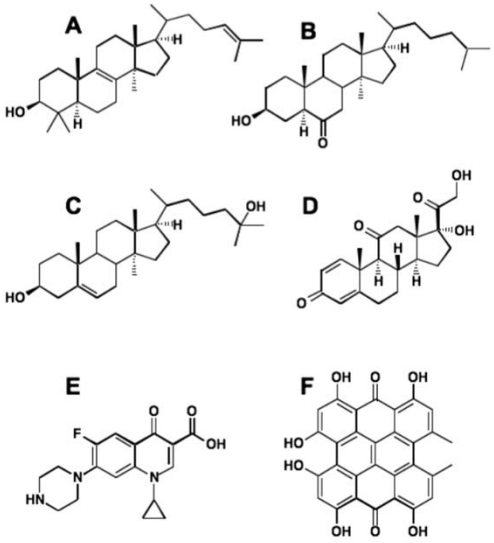


Testosterone



17β-estradiol

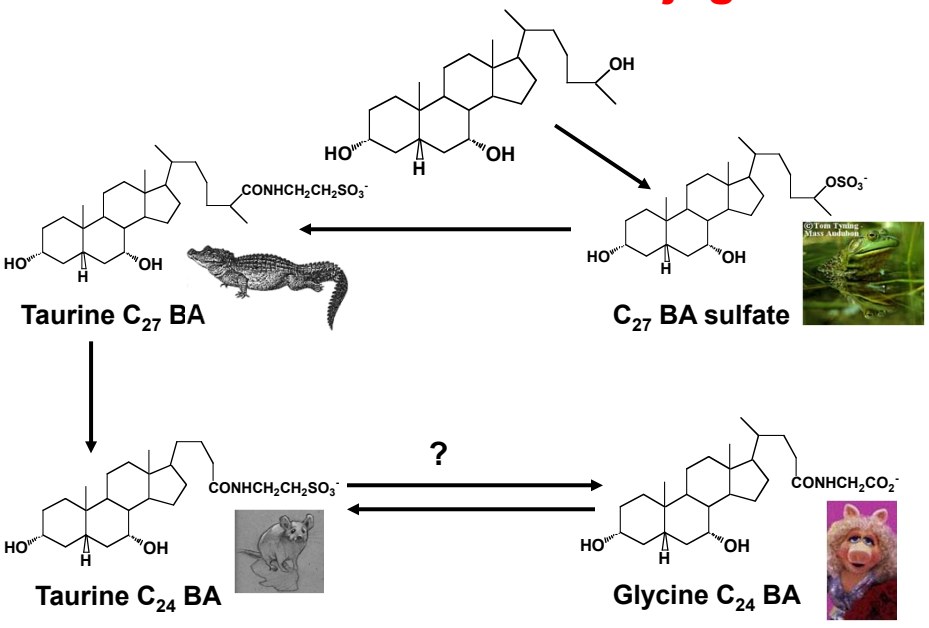
Importance of sterols and other compounds in lens cataracts



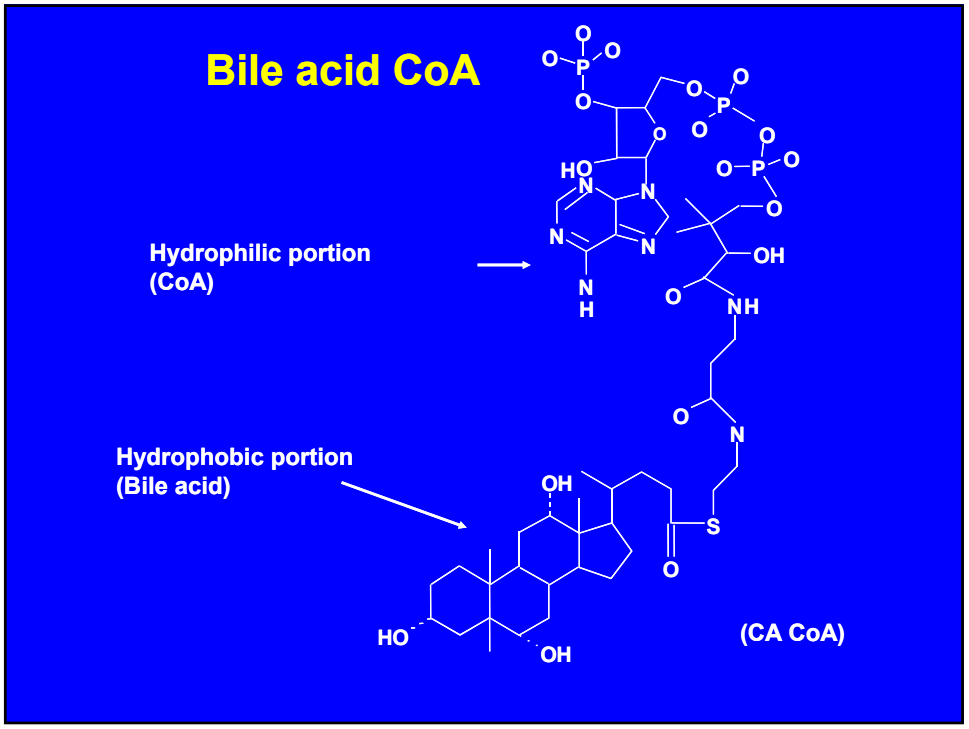
Structures A, B and C (all sterols) have recently been shown to have the property of "dissolving" lens cataracts. Cholesterol, on the other hand, has no effect. Other sterols observed in *cerebrotedinous xanthamatosis* promote cataracts.

D, E and F all promote lens cataracts. D is prednisone (an anti-inflammatory steroid), E is ciprofloxacin (an antibiotic) and F is hypericin from the botanical, St. John's wort.

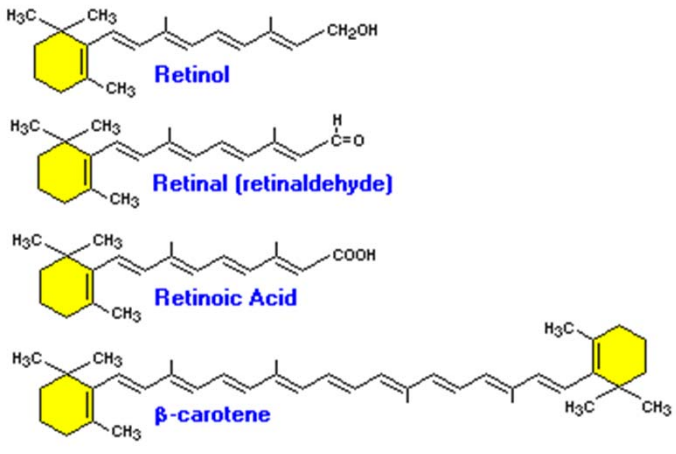
Evolution of bile acid conjugation



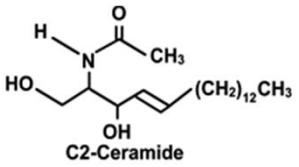
Bile acid CoA



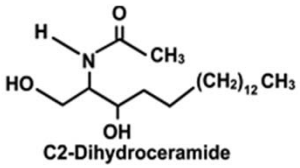
Vitamins



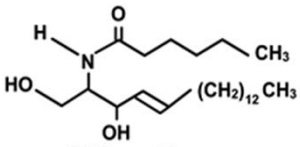
Phospholipids



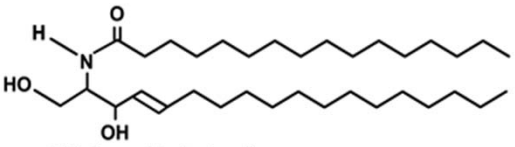
C2-Ceramide



C2-Dihydroceramide

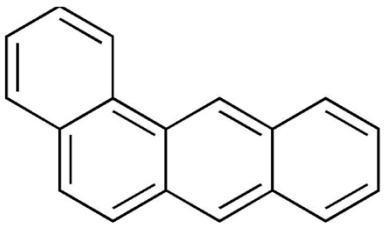


C6-Ceramide



C16-Ceramide (natural)

Hydrocarbons



Benz[a]anthracene

Cetyl palmitate

Solubilities of the metabolites

- Those in biological fluids are "in solution", but may not be soluble in water or methanol alone
  - Are glucose or amino acids soluble in methanol?
  - Are cholesterol esters in plasma soluble in methanol or water?
    - If a metabolite binding protein is precipitated by methanol, does the metabolite still bind to it?
  - Does pH have an effect on solubility?