

GBS 748

The Chemistry of the metabolome

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What is a metabolite?

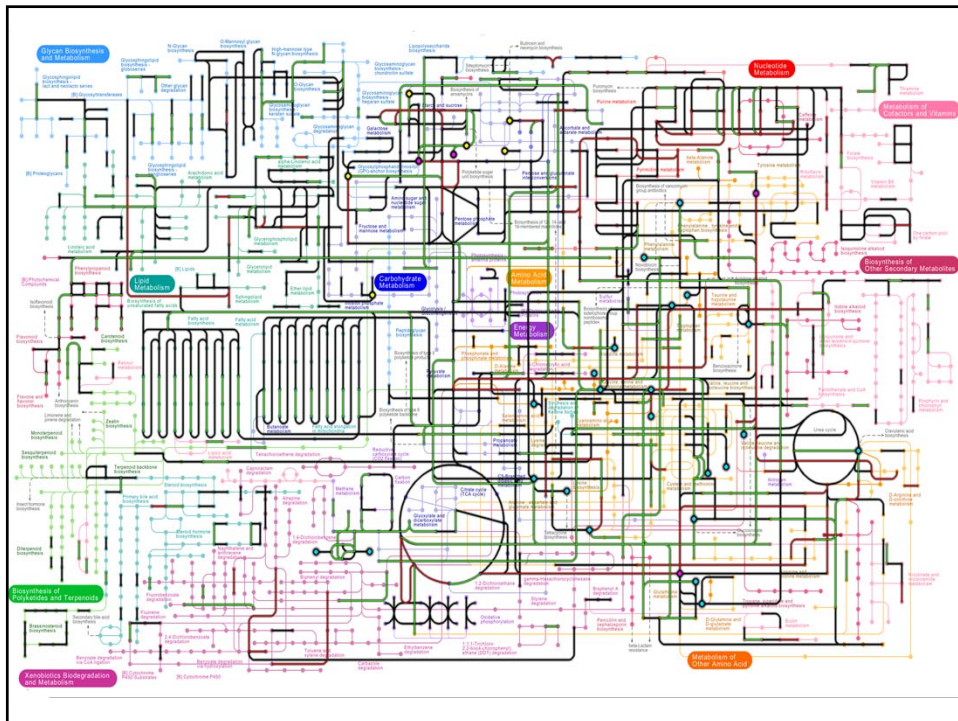
- In the context of metabolomics, it is *any compound of any origin that has a molecular weight <1,500 Da that is detected in the biological system being studied*
- This is an arbitrary definition

The metabolome is more than what's in textbooks

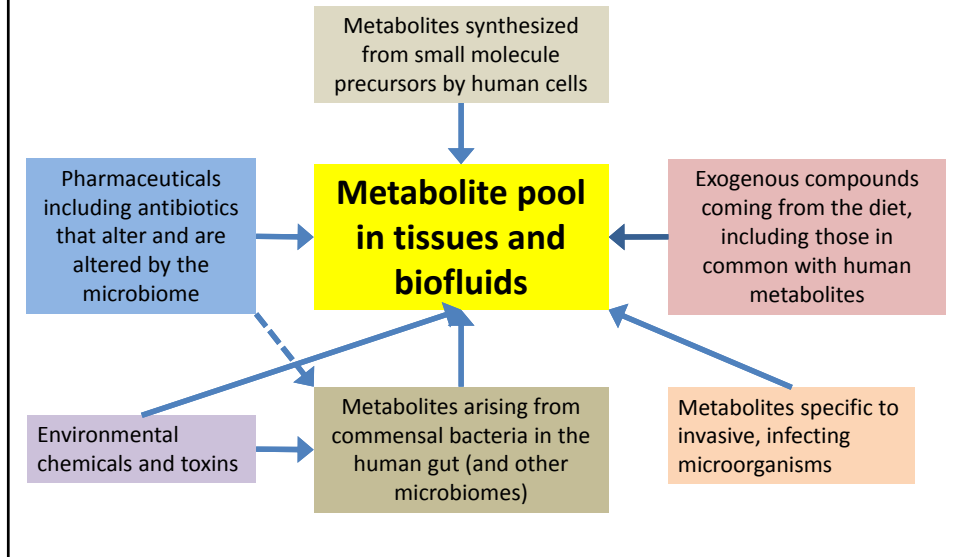
Metabolites synthesized from small molecule precursors by human cells



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

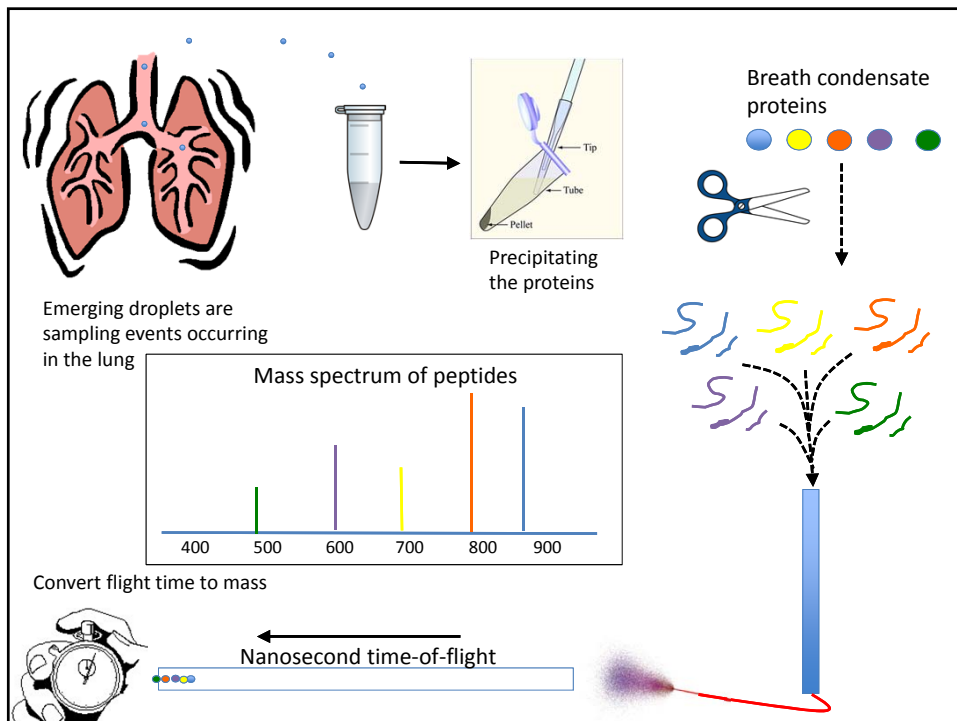
- **In breath**
 - H₂ from reductive anerobic bacteria
 - Lactose-intolerant
 - Measure of gut transit (typically 4-6 hours)
 - CO₂
 - From all carbon-containing substrates
 - From specific ¹³C-labelled substrates
 - Acetone (in diabetics)
 - Trimethylamine
 - From fish or FMO3-deficient subjects

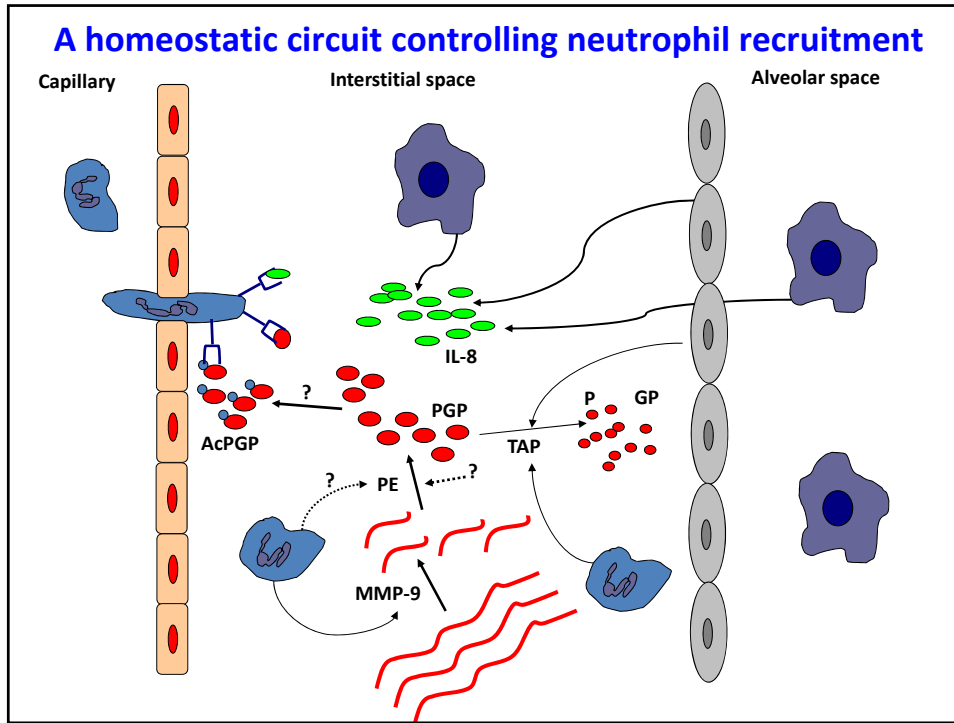
Gases and volatiles

- **Sweat gland**
 - Sweaty socks syndrome
 - Isovaleric acid (isoleucine metabolism)
- **Flatulence**
 - Mostly gases (H₂, CO₂ and H₂S), 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

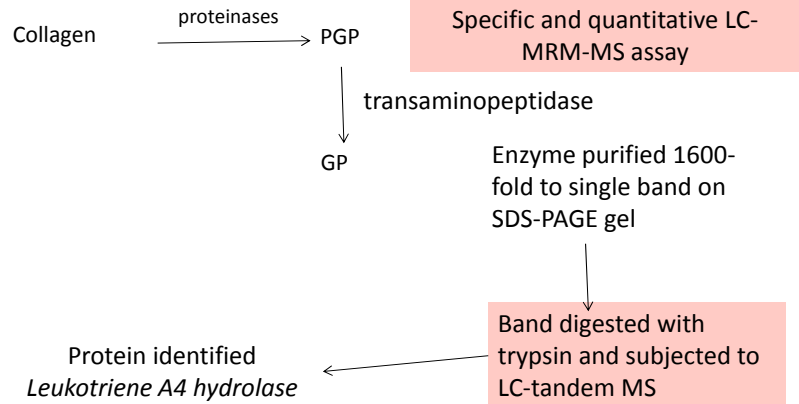




PGP is a common peptide in human collagen

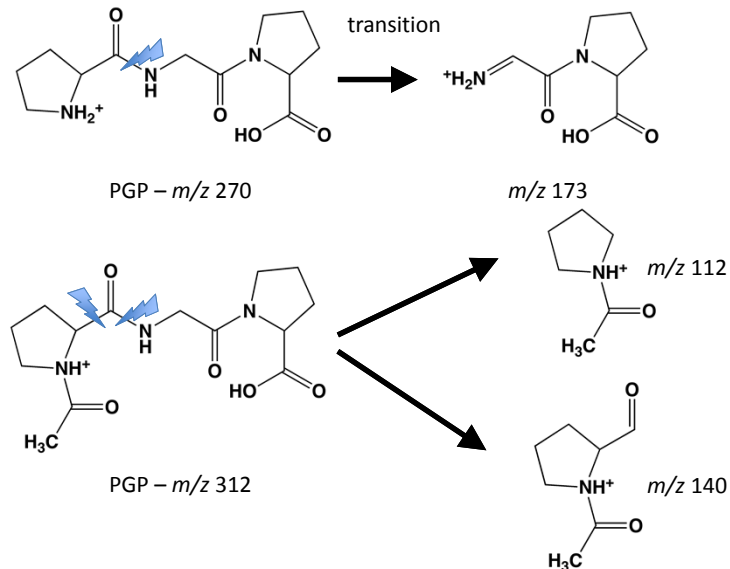
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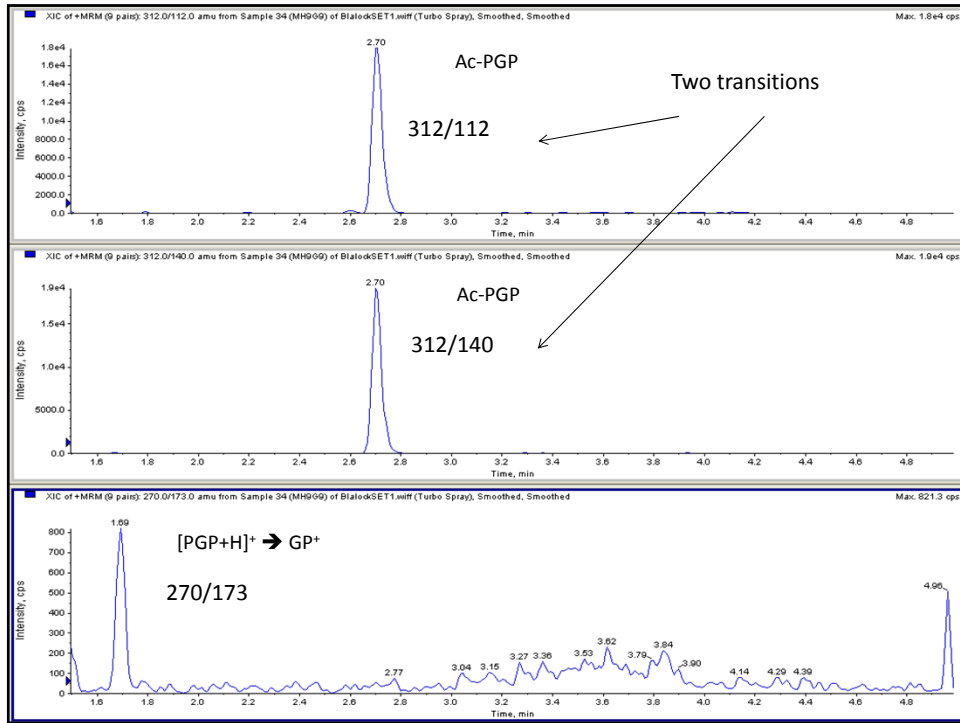
Mass spec contribution to PGP story



(Robert Snelgrove et al. Science, 2010)

Measuring PGP and acetyl-PGP





Metabolopeptidomics or peptidometabolomics

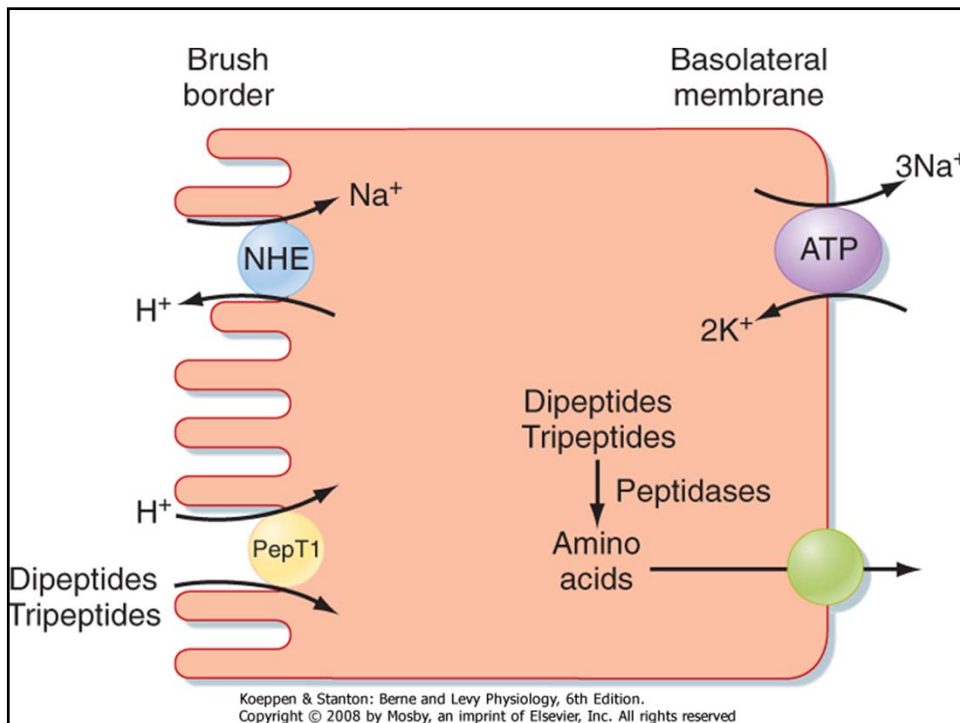
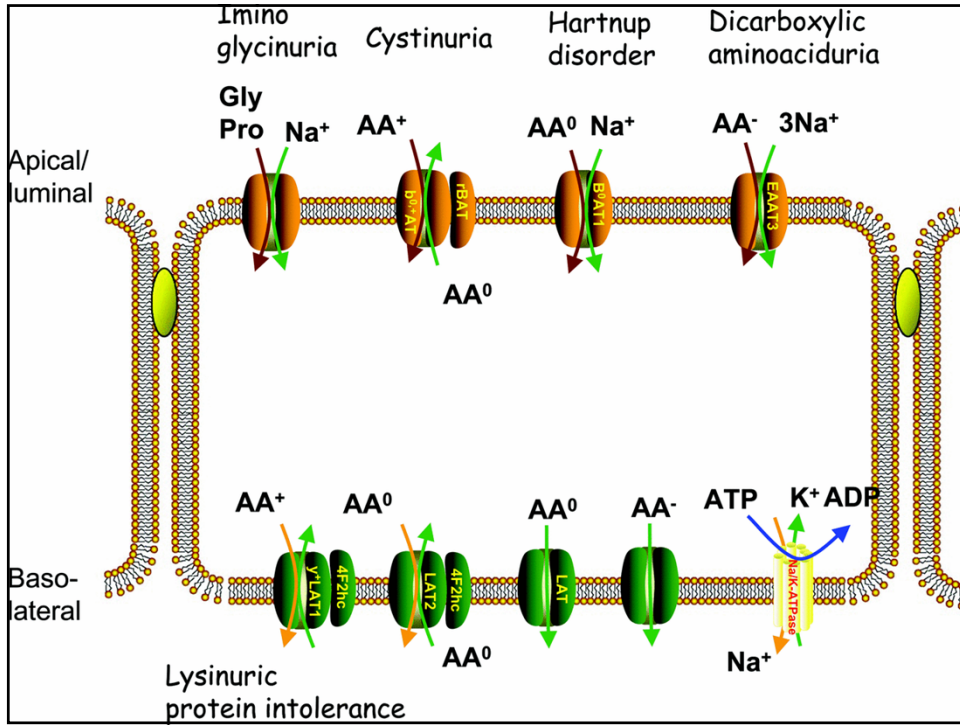
- Are peptides metabolites?
- Are the tripeptides real?
Or is their mass simply coincident with the empirical formula of another metabolite?

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



Koepfen & 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**

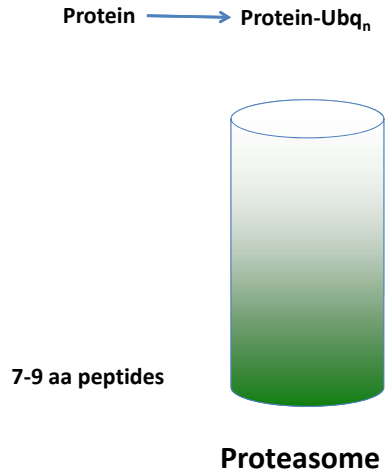
**Deficiencies in the peptidases could lead to
food and bacterial peptides entering the
systemic circulation**

Can tripeptides have biological activity?

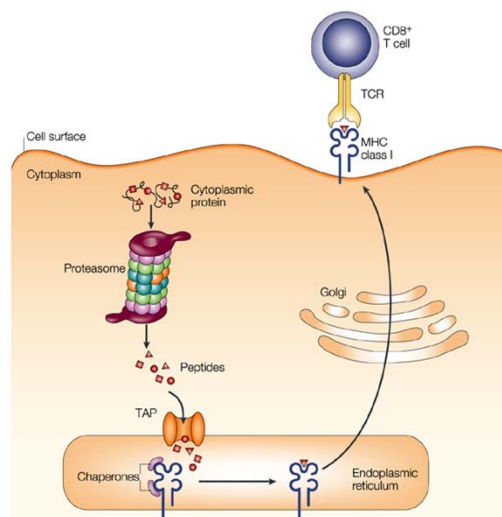
- **For toxicologists, there is one very familiar tripeptide without whom, I would not be giving this talk, or you to listen to it.**
- **Glutathione (GSH) – glutamyl-cysteinyl-glycine**
 - GSH reacts with free radicals to generate GSH conjugates and therefore protects many organs
- **It is synthesized 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 (escapes?)
- Lysosomes
- Autophagosome
- Neutrophil attack
- Other proteases (in renal tubules?)



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



Nature Reviews | Immunology

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

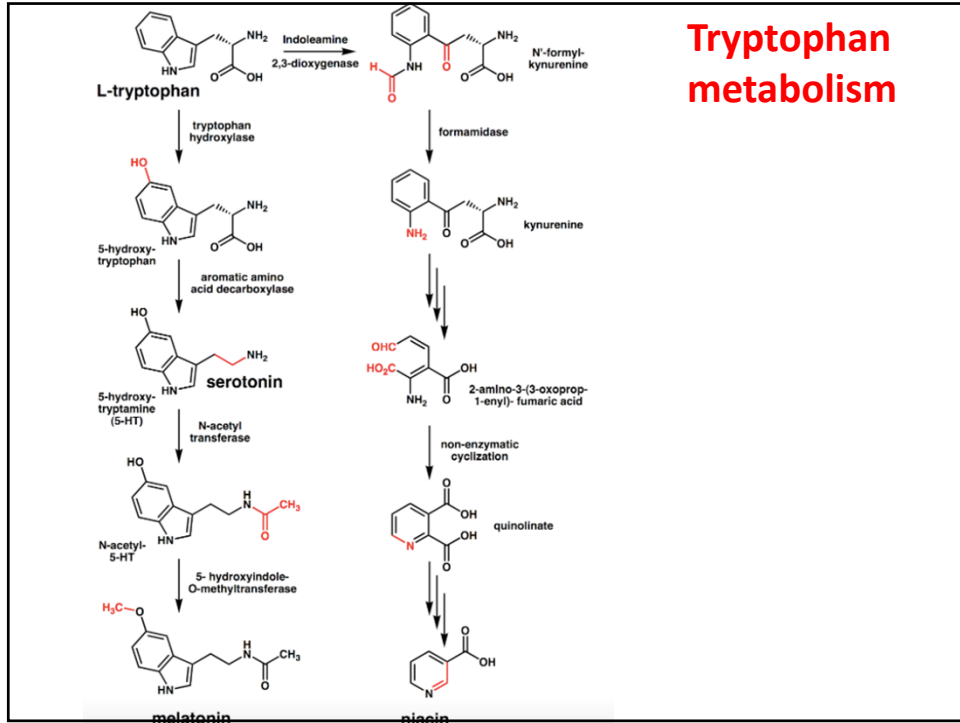
How could we isolate amino acids?

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



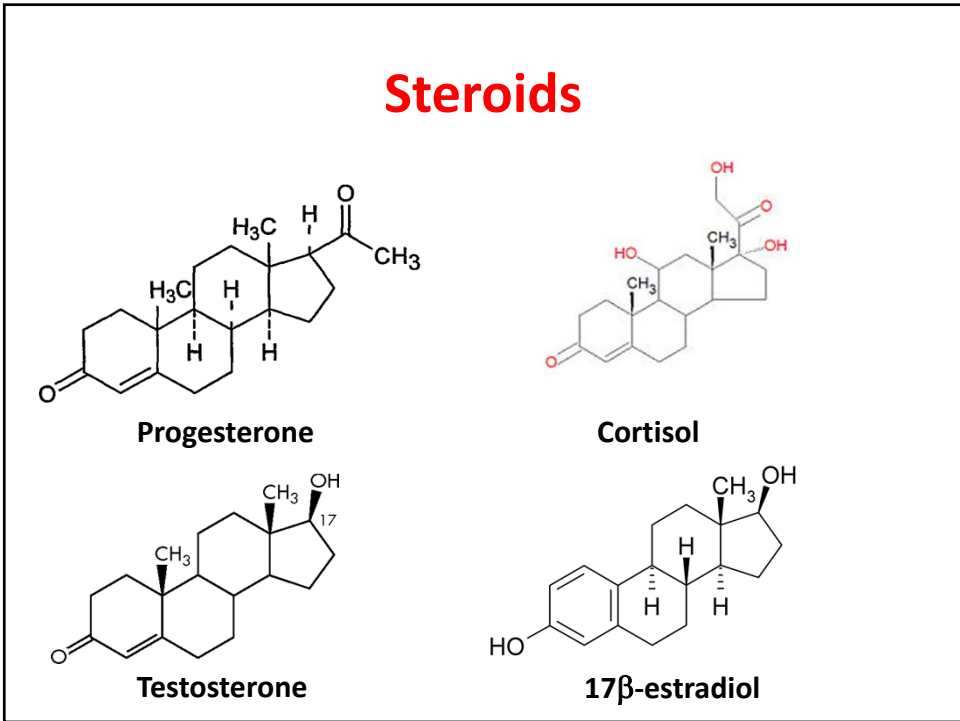
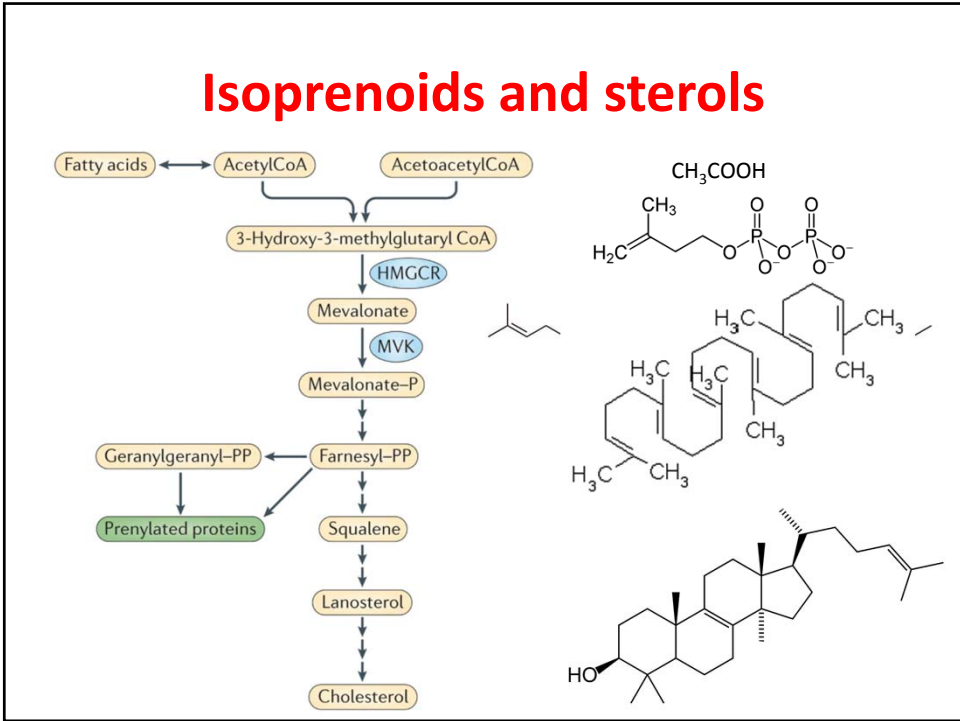
AG-50

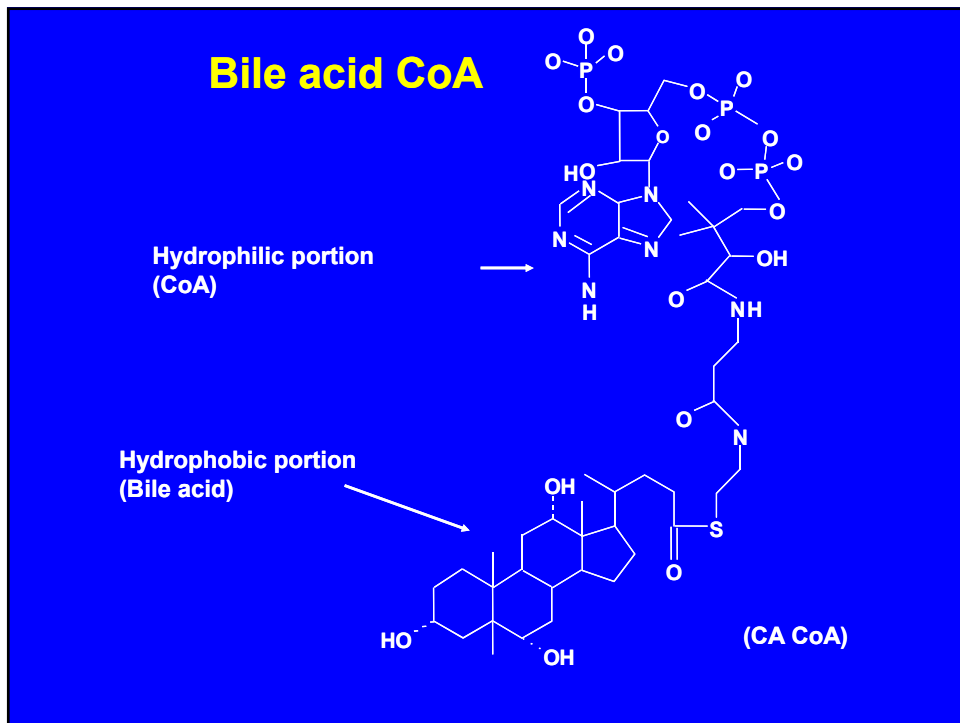
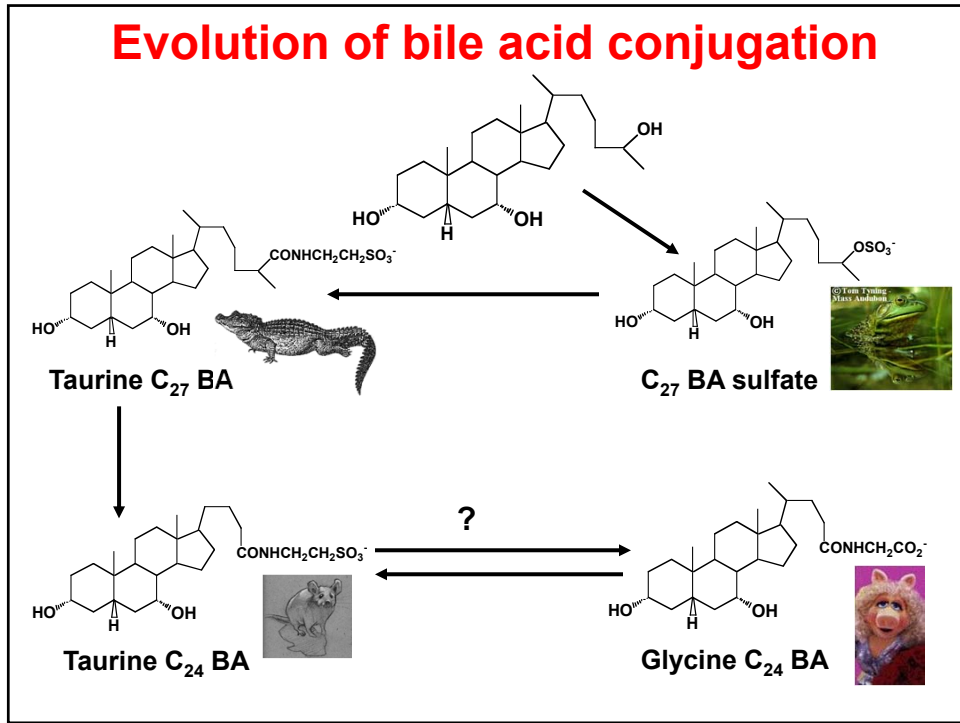
- Can be eluted with ammonium hydroxide (mass spec compatible)



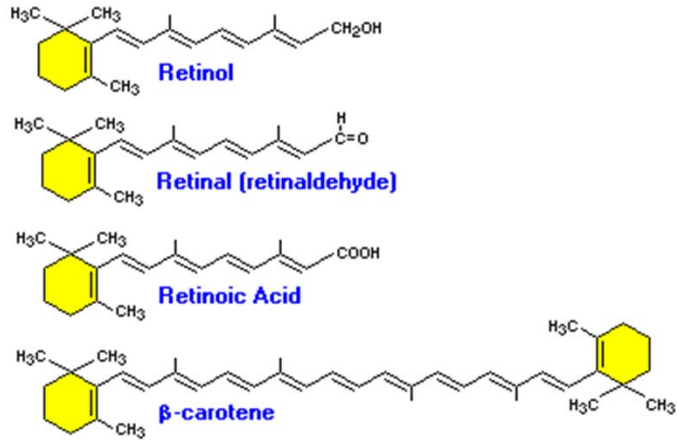
Hydrophobic metabolites

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

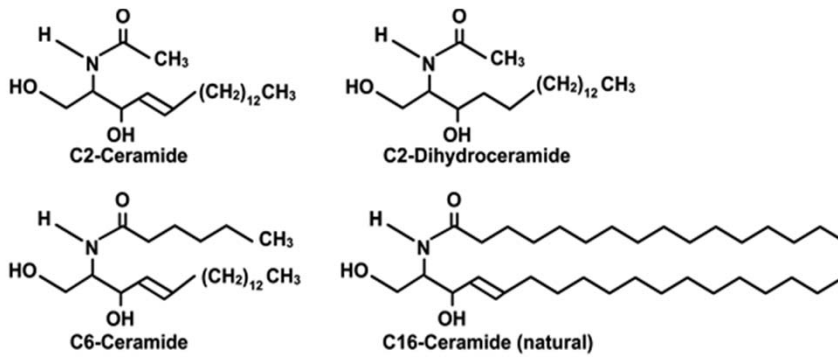




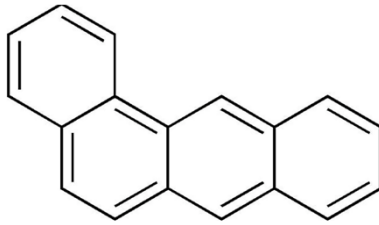
Vitamins



Phospholipids



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?