



*Knowledge that will change your world*

## **The Chemistry of the metabolome**

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### **What is a component of the metabolome?**

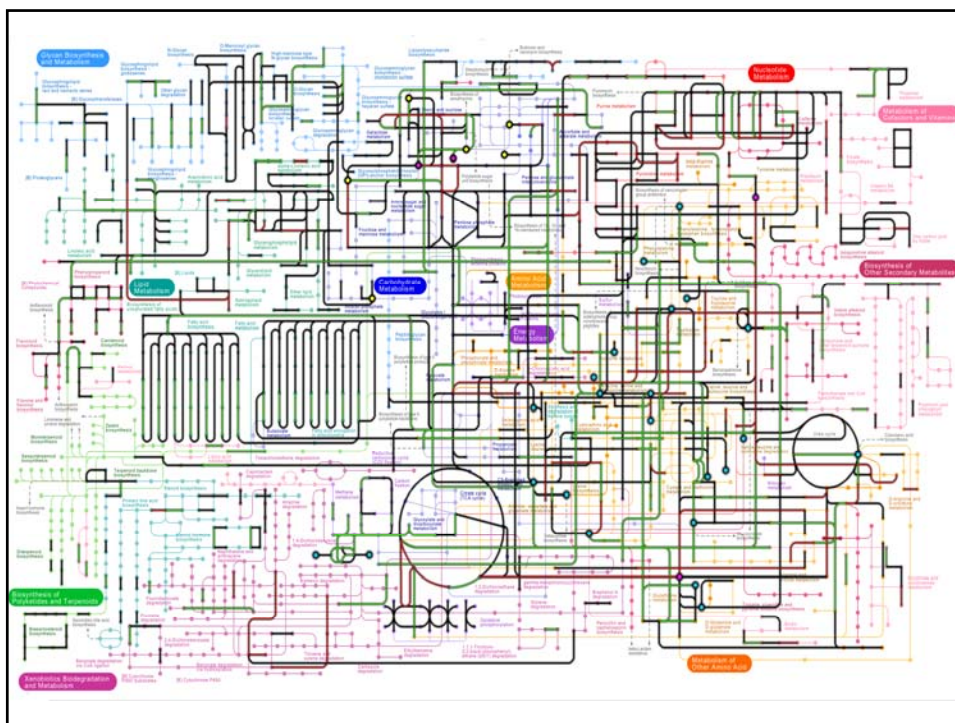
- In the context of metabolomics, it is *compound of any origin that has a molecular weight <1,500 Da that can be detected in the biological system being studied*
- This is an arbitrary definition

## The metabolome is more than what's in textbooks

Metabolites synthesized from small molecule precursors by human cells

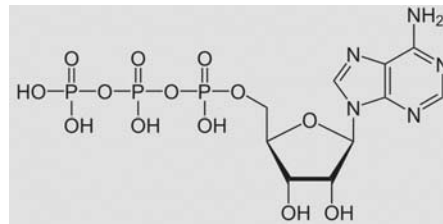


**Metabolite pool in tissues and biofluids**

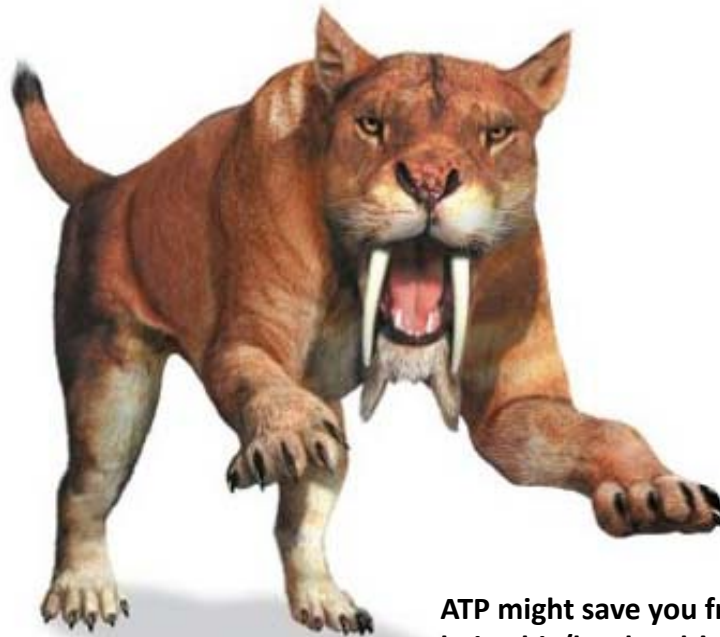
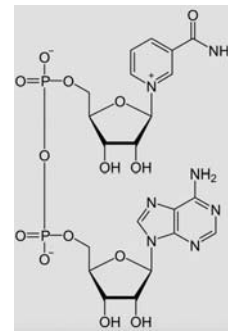


## Critical metabolites

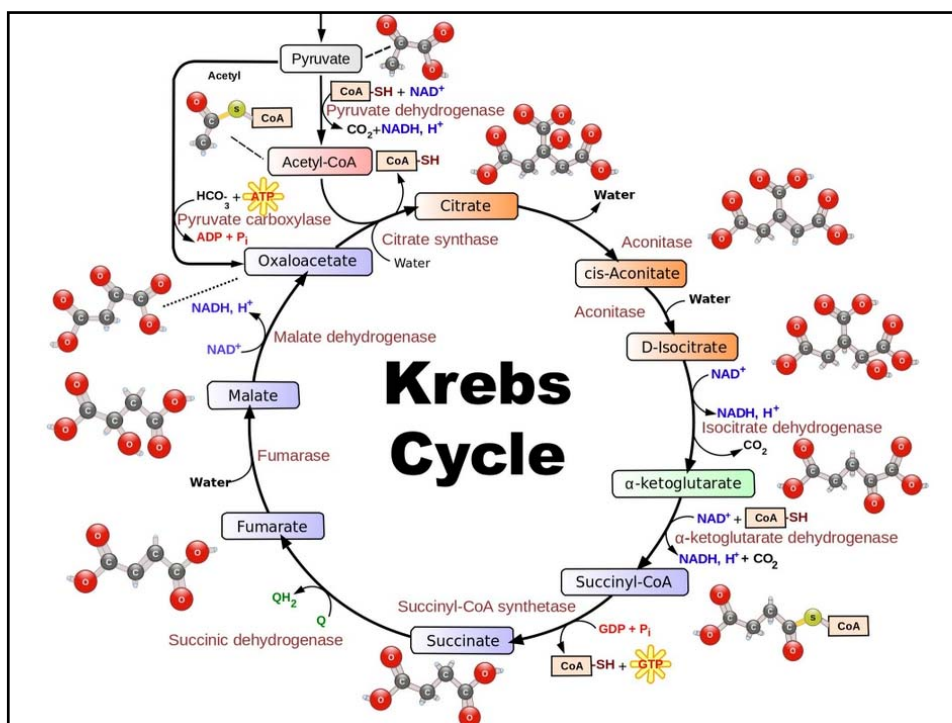
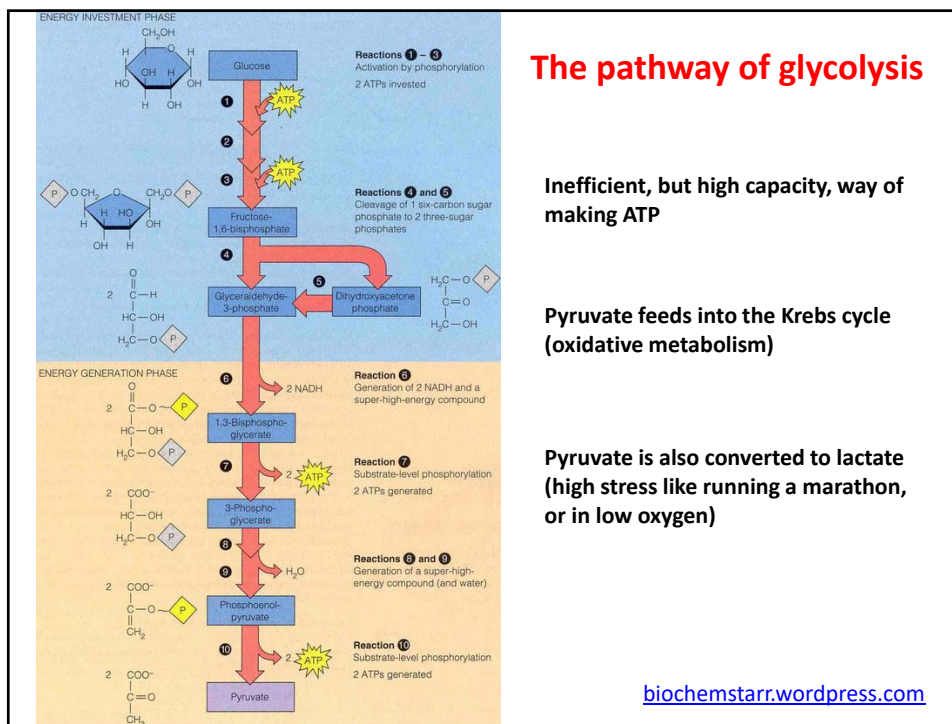
ATP: adenosine-5-triphosphate



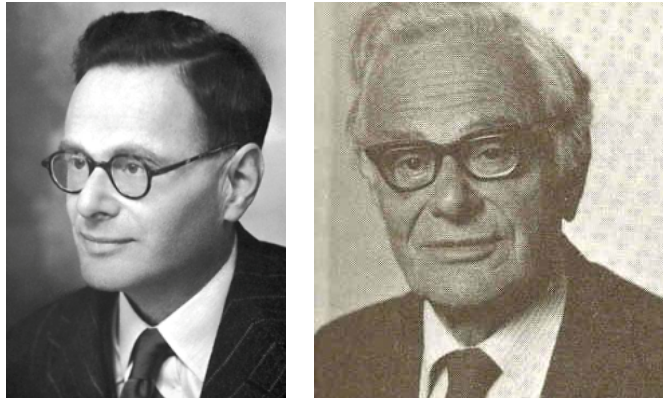
NAD<sup>+</sup>/NADH: nicotinamide adenine dinucleotide



ATP might save you from being his/her lunch!

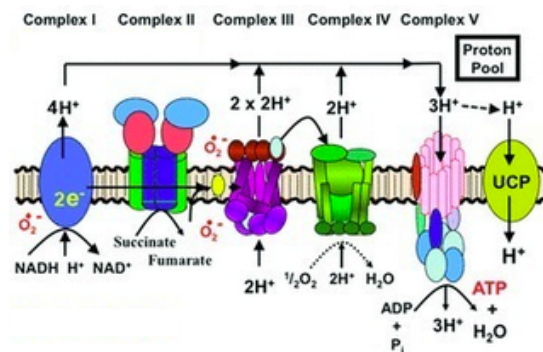


## Sir Hans Krebs



Had the pleasure as a graduate student of introducing him at a seminar

## Mitochondrial oxidative phosphorylation

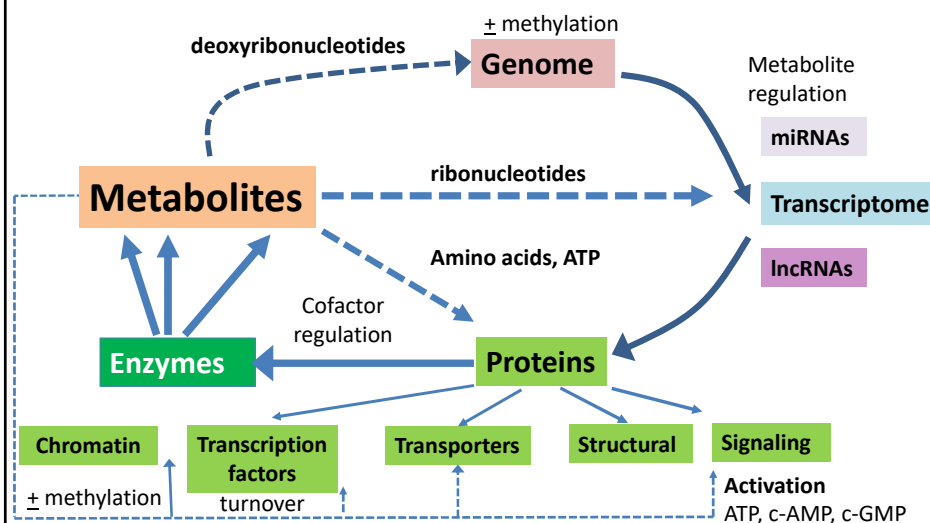


NADH from the Krebs cycle, as well as succinate, generate a proton ( $H^+$ ) gradient (upper region) that drives rotation of one of the subunits of ATP synthase. This exposes the catalytic domain of this enzyme and makes ATP.

## Understanding metabolites

- Metabolites represent the *action items* that come from gene expression and protein activity
  - They are found in the same range of concentrations as drugs
  - Metabolites ( $\mu\text{M}$  or  $\text{mM}$ ) (acetyl CoA, ATP, SAdMet,  $\alpha\text{KG}$ ) are regulators of epigenetics
  - Bile acids ( $\mu\text{M}$ ) are the natural ligands of FXR and LXR
  - Other metabolites ( $\text{pM}$  or  $\text{nM}$ ) may be exquisite physiological regulators of kidney function (prostaglandins,  $\text{F}_2$ -isoprostanes)
- Studying the metabolome requires multiple levels of science from the analytical to the physiologic to the computational

### Metabolites are associated with every aspect of cellular events

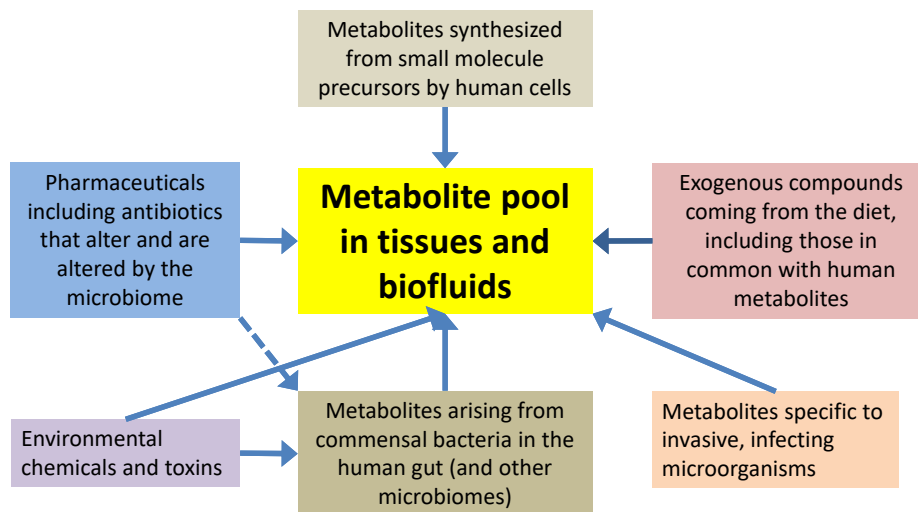


## Metabolism and time

- Not only should metabolites appear in the right place, there is also the question of the importance of the timescale
- Metabolism defects in the heart may be only seconds away from death – rogue waves in metabolism??
- Irreversible damage to the brain may occur in minutes
- Go/No-Go decisions for a cell to divide or apoptose may occur in tens of mins



## The metabolome is more than what's in textbooks



## Overview of metabolome chemistry

Metabolites encompass an enormous range of chemistries

- **Gaseous**
  - H<sub>2</sub>, H<sub>2</sub>S
- **Volatile**
  - Butyric acid, acetone, skatole
- **Hydrophilic (water-loving)**
  - Glucose
- **Charged-positive/negative**
  - Amino acids, nucleotides, organic acids, amines
- **Hydrophobic (fat-loving)**
  - Lipids, steroids, hydrocarbons

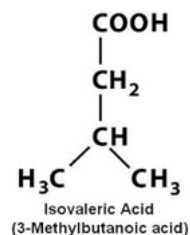
## Gases and volatiles

- **In breath**
  - H<sub>2</sub> from reductive anerobic bacteria
    - Lactose-intolerant
    - Measure of gut transit (typically 4-6 hours)
  - CO<sub>2</sub>
    - From all carbon-containing substrates
    - From specific <sup>13</sup>C-labelled substrates
  - Acetone (in diabetics)
  - Trimethylamine
    - From fish, or flavin monooxygenase (FMO3)-deficient subjects

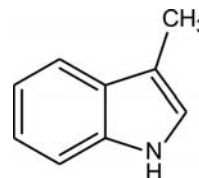


## Gases and volatiles

- **Sweat gland**
  - **Sweaty socks syndrome**
    - **Isovaleric acid** (leucine metabolism)
    - **Caused by bacteria or enzyme defect**



- **Flatulence**
  - **Mostly gases (H<sub>2</sub>, CO<sub>2</sub> and H<sub>2</sub>S), but with volatiles produced by colonic bacteria (**skatole**, from the amino acid tryptophan)**

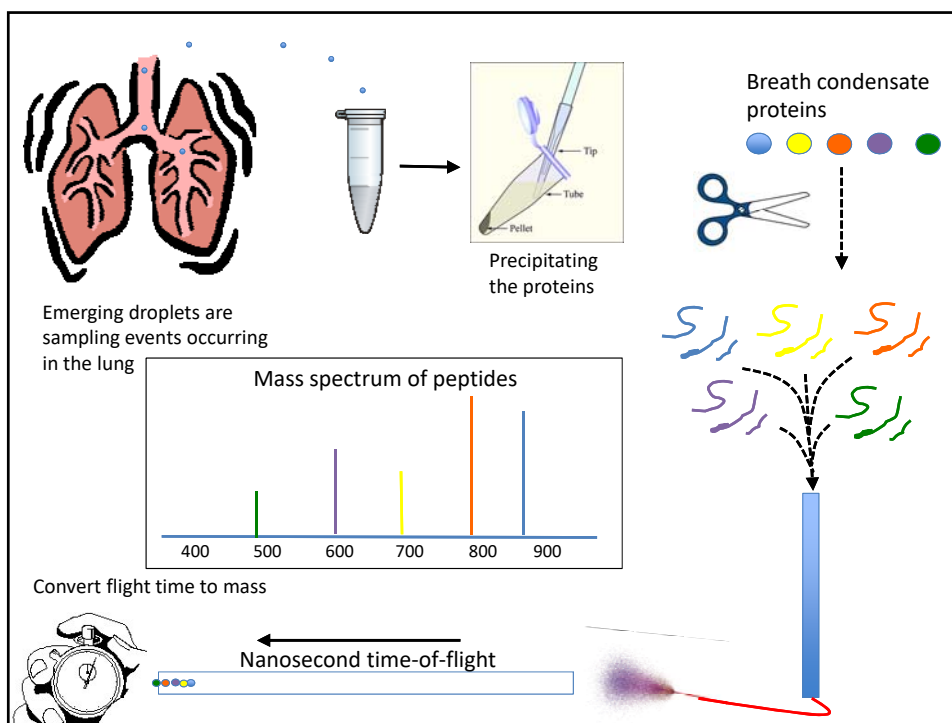


## Other volatiles

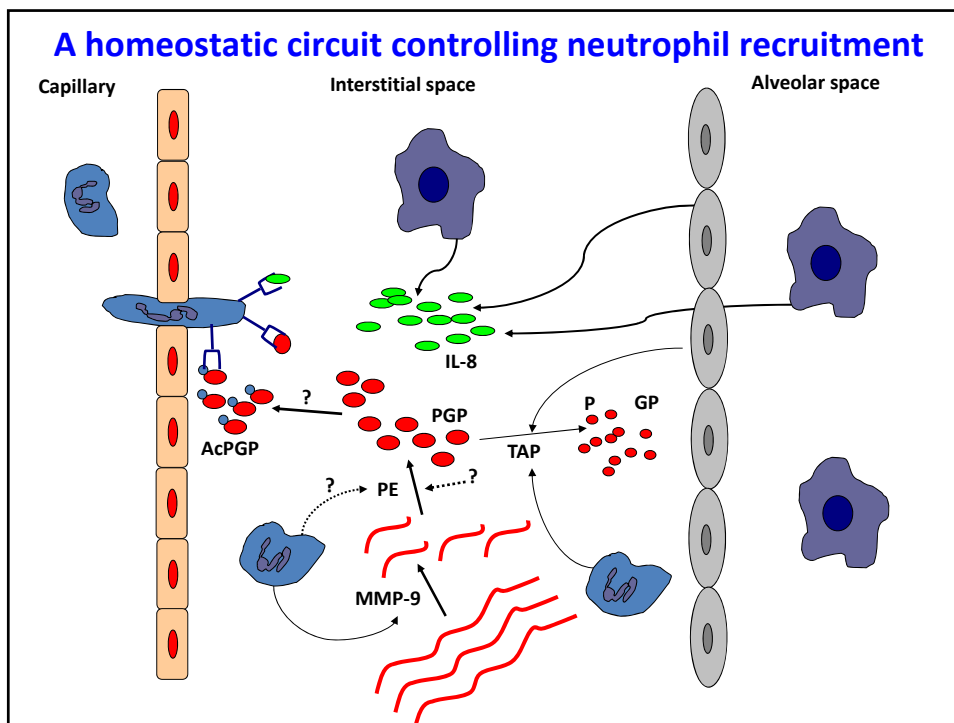
- **Short chain, unsubstituted fatty acids**
  - **Formic, acetic, propionic, butyric acids**
- **Will evaporate in the acidic form**
  - **Formic acid, b.p. 101°C**
  - **Acetic acid, b.p. 118°C**
  - **Propionic acid, b.p. 141°C**
  - **Butyric acid, b.p. 163.8°C**
  - **Isobutyric acid, b.p. 155°C**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

## 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 in 5-8 min



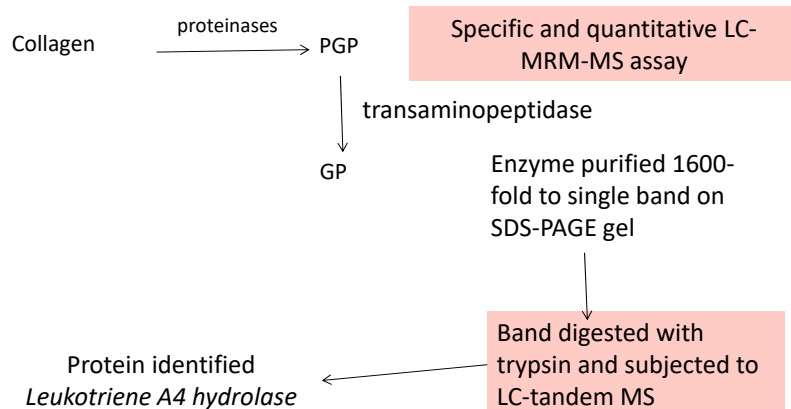
## Certain metabolites are peptides



## PGP is a common peptide in human collagen

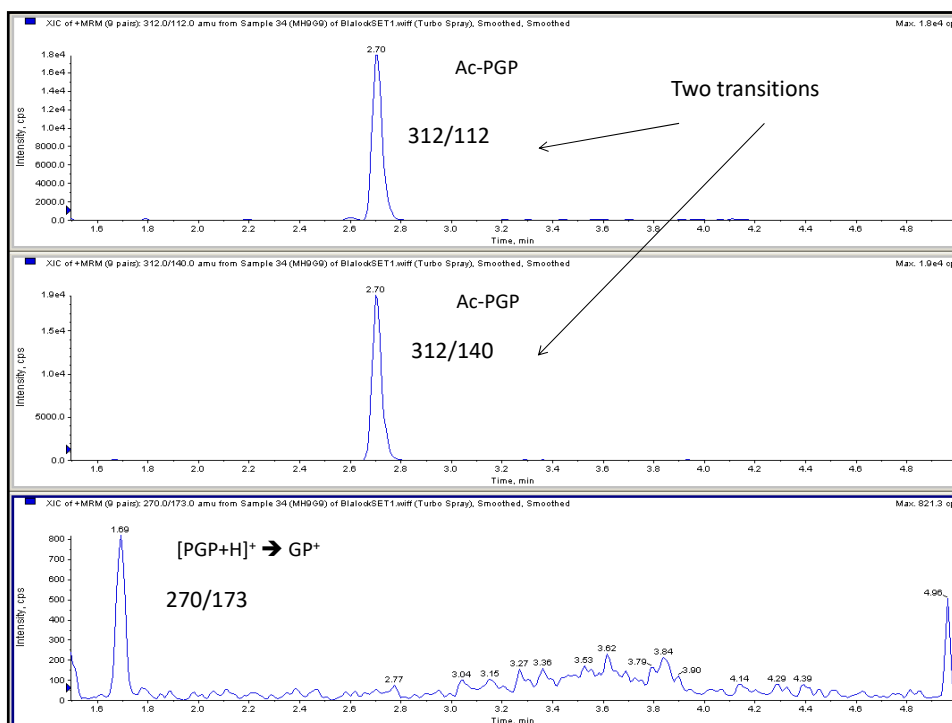
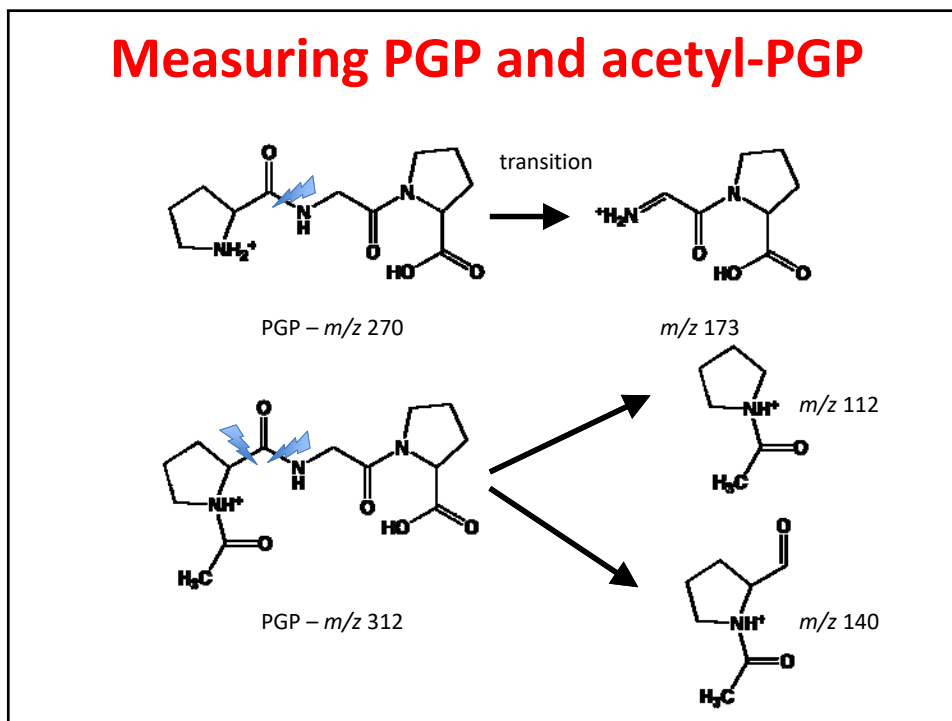
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 GPRGPAGPPGRDGI PGQPGLPGPP**PGPPGPPGPP**PGLGGNFAPQLSYGYDEKSTGGISV**PGP**  
 MGPSGPRGL**PGPPGAPGP**QGFQGPPEPEGEPGASGPMGPRGP**PGPP**PGKNGDDGEAGKPGR  
 PGERGP**PGP**QGARGLPGTAGLPGMKGHRGFSGLDGAKGDAGPAGPKGEPGSPGENGAPGQ  
 MGPRGLPGERGRPGAP**PGP**AGARGNDGATGAAGP**PGP**TGPAGPPGFP GAVGAKGEAGPQGP  
 RGSEGPQGV RGE**PGPPGP**AGAAGPAGNPGADGQPGAKGANGAPGIAGAPGFPGARGPSG  
 QGPGGP**PGP**KGNSGEPGAPGSKGDTGAKGE**PGP**VGVQGP**PGP**AGEEGKRARGE**PGP**TGL  
**PGP**PPERGGPSRGPFGADGVAGPKGPAGERGS**PGP**AGPKGSPGEAGRPEAGLPGAKGL  
 TGS PGS**PGP**DGKTGP**PGP**AGQDGR**PGPPGP**PGARGQAGVMGF**PGP**KGAAGEPGKAGERGV  
**PGP**PGAVGPAKDG EAGA QGP**PGP**AGPAGERGEQGPAGSPGFQGL**PGP**AGPPGEAGKPG  
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 TGNLKKALLLQGSNEIEIRAEGNSRFTYSVTVDGCTSHTGAWGKTVIEYKTTKTSRLPII  
 DVAPLDV GADQEF GFDVGPVCFL

## 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?**

**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**

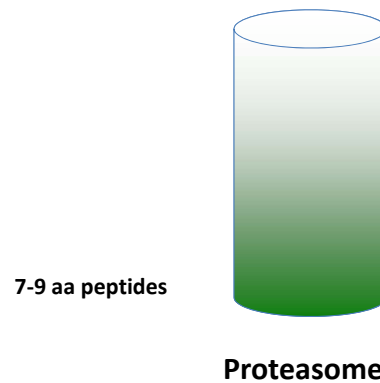
### **Can tripeptides have biological activity?**

- **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?)
- Foreign antigens hydrolyzed and presented on surface of cells

Protein  $\longrightarrow$  Protein-Ubq<sub>n</sub>



## Hydrophilic metabolites


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



## Organic acids

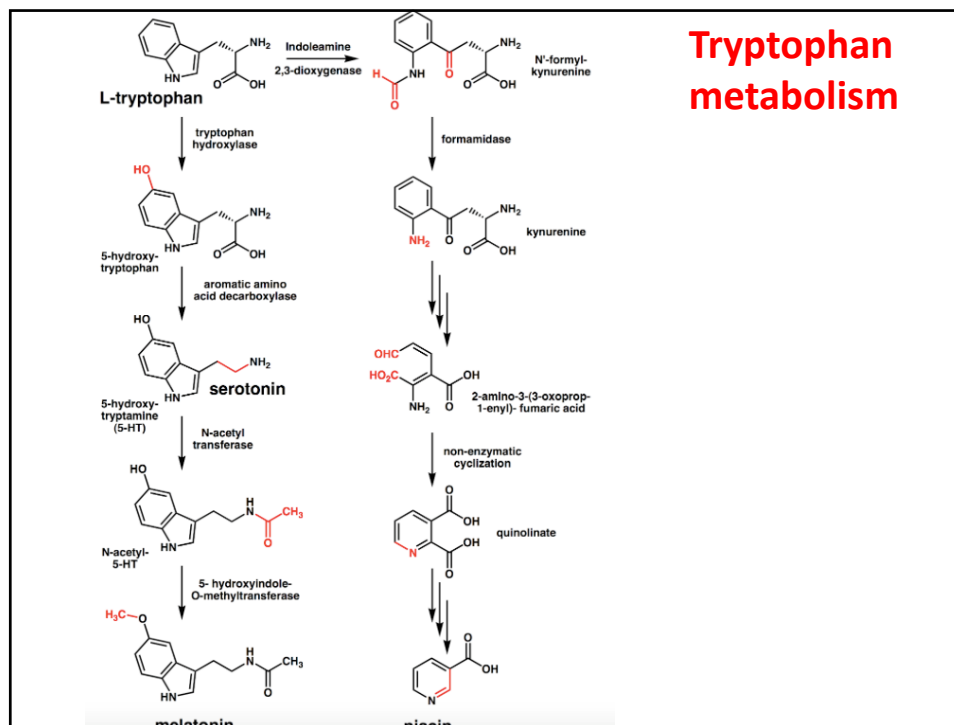
- Besides the short chain fatty acids mentioned earlier, there are many organic acids representing important cellular pathways
  - Glycolytic intermediates
    - Glucose-1-P, Glucose-6-P, Fructose-6-P, Fructose-1,6-DP, Glyceraldehyde-3-P, Dihydroxyacetone-P, Glycerate-3-P, Phosphoenol-P, Pyruvate, Lactate
  - Krebs cycle
    - Citrate, cis-Aconitate, Iso-Citrate,  $\alpha$ -ketoglutarate, Succinate, Fumarate, 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  $\alpha$ -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
- $\beta$ -Alanine is formed from uracil
  - 5-fluorouracil (anticancer drug) is converted to 2-fluoro- $\beta$ -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<sup>+</sup> form



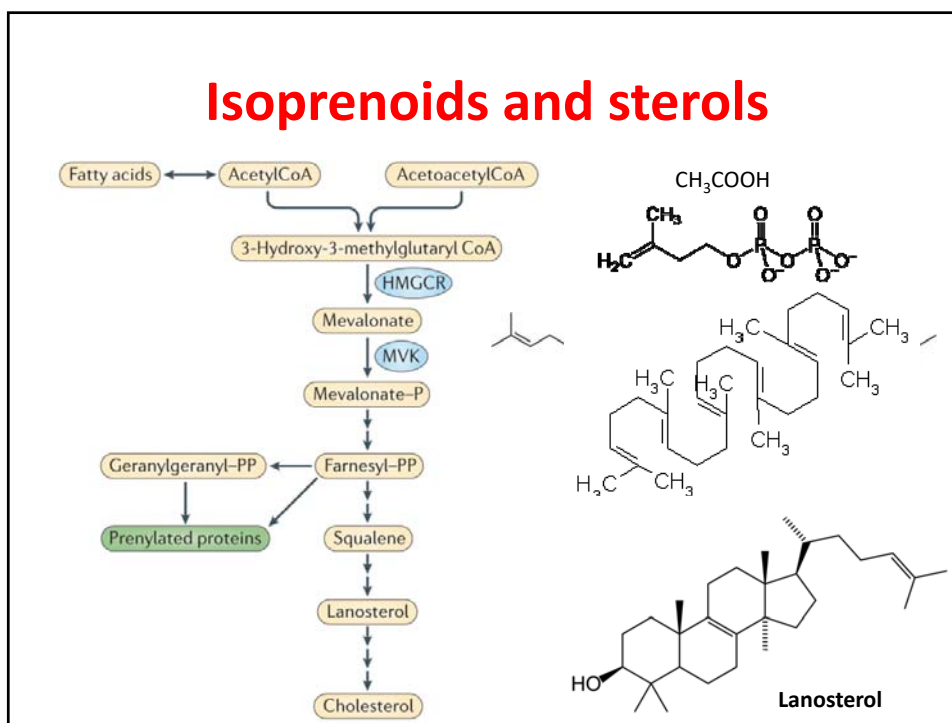
AG-50

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

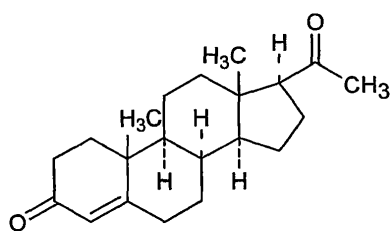
## Hydrophobic metabolites

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

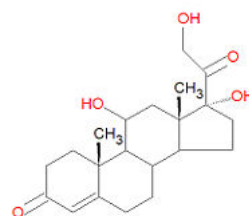
## Isoprenoids and sterols



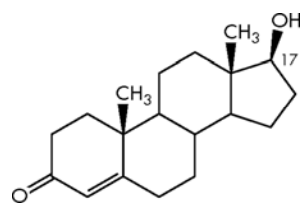
## Steroids



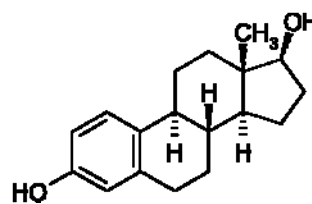
Progesterone



Cortisol

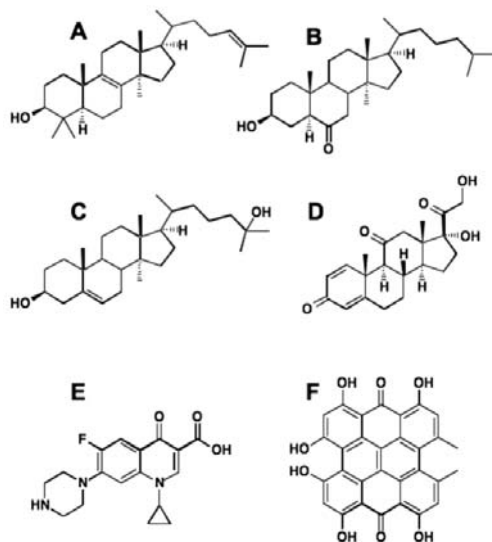


Testosterone



17β-estradiol

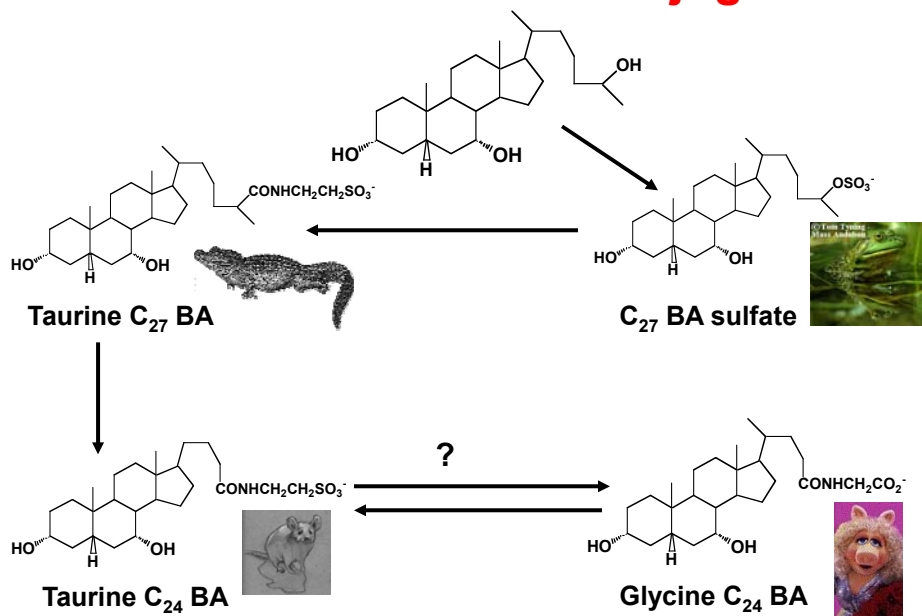
## Importance of sterols and other compounds in lens cataracts



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 *cerebrotendinous xanthomatosis* 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.

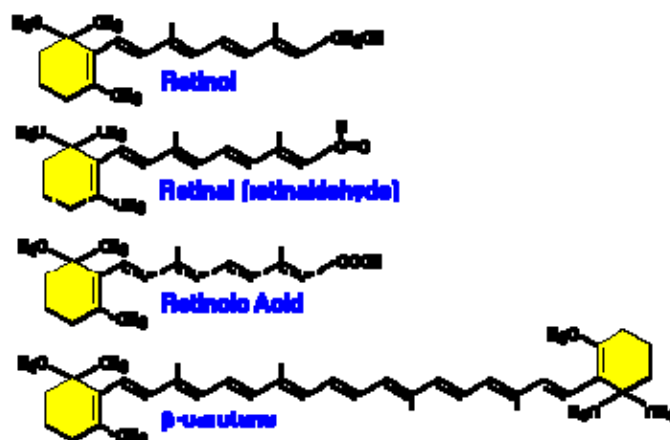
## Evolution of bile acid conjugation



## The vitamins

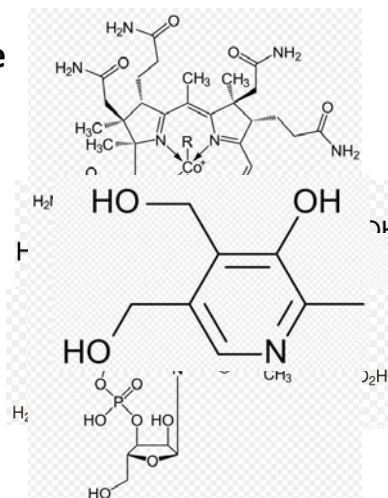
Lack of these leads to serious illness, but not death

## Vitamin A



## Vitamin B

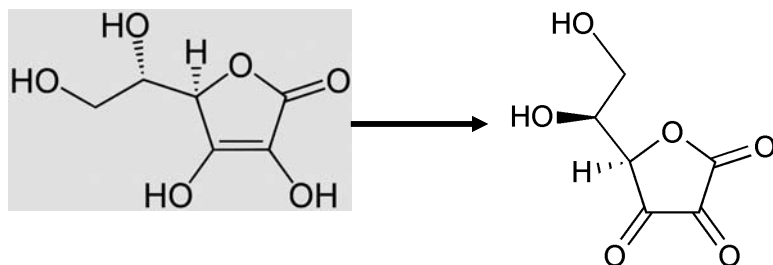
- They are all water-soluble
  - Vit B<sub>1</sub> – thiamine
  - Vit B<sub>2</sub> – riboflavin
  - Vit B<sub>3</sub> – niacin
  - Vit B<sub>5</sub> – pantothenic acid
  - Vit B<sub>6</sub> – pyridoxine
  - Vit B<sub>7</sub> – biotin
  - Vit B<sub>9</sub> – folic acid
  - Vit B<sub>12</sub> – cobalamins



They are not made by human enzymes and if deficient in the diet cause disease

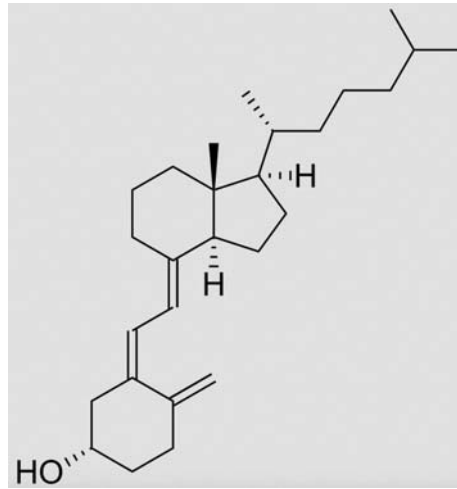
## Vitamin C

- Ascorbic acid



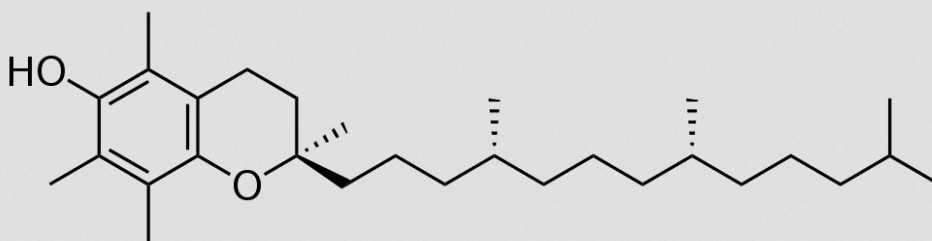
dehydroascorbic acid

## Vitamin D



In fish, supplemented in milk, made in skin by UV light

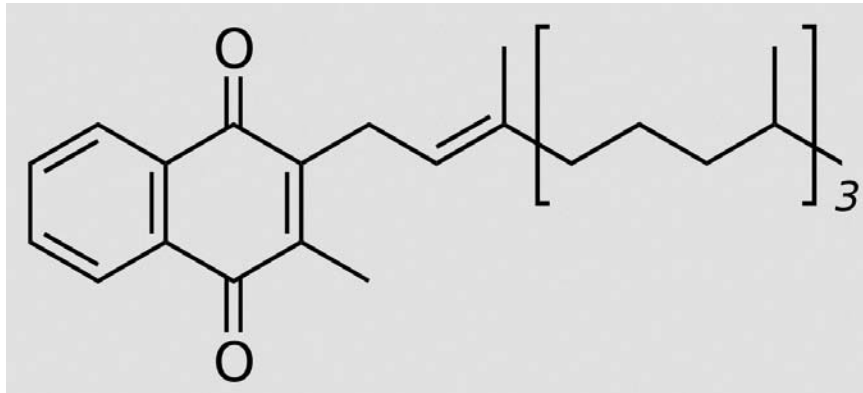
## Vitamin E



Found in oils from plants

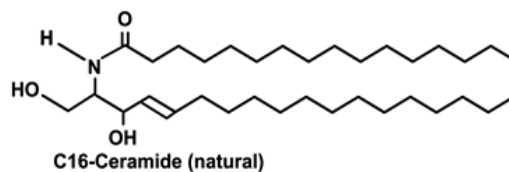
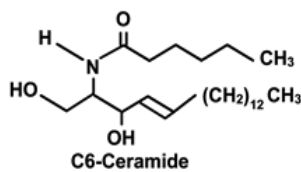
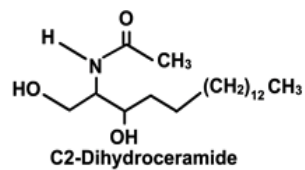
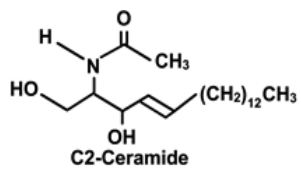


## Vitamin K

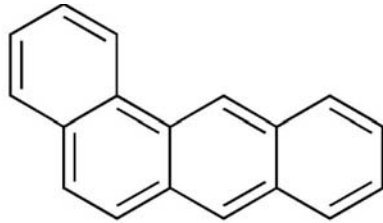


Is an anticoagulant – needed to stop bleeding

## Phospholipids



## Hydrocarbons



**Benz[a]anthracene**  
In smoke from barbecued meat



**Cetyl palmitate**  
In hair shampoo

## 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?