Knowledge that will change your world

The Chemistry of the metabolome

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Overview

- **The major energy producing pathways**
  - Glycolysis, Krebs Cycle, mitochondria
- **Critical importance of metabolites for life**
- **Complexity of the metabolome**
- **Importance of bacteria**
- **Diversity of metabolome chemistry**
  - From gases to earwax, even peptides
  - Vitamins, steroids and lipids

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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**

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The metabolome is more than what's in textbooks

Metabolites synthesized from small molecule precursors by human cells

↓

Metabolite pool in tissues and biofluids

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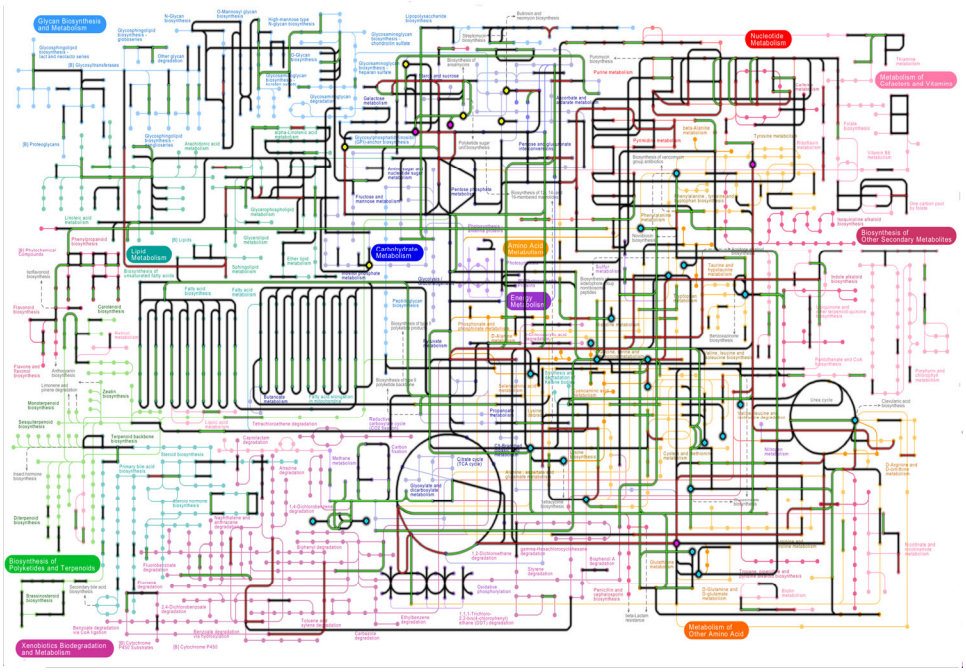

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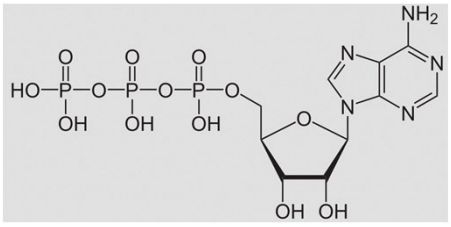
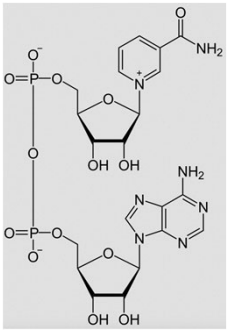
Critical metabolites

ATP: adenosine-5-triphosphate



NAD⁺/NADH: nicotinamide adenine dinucleotide



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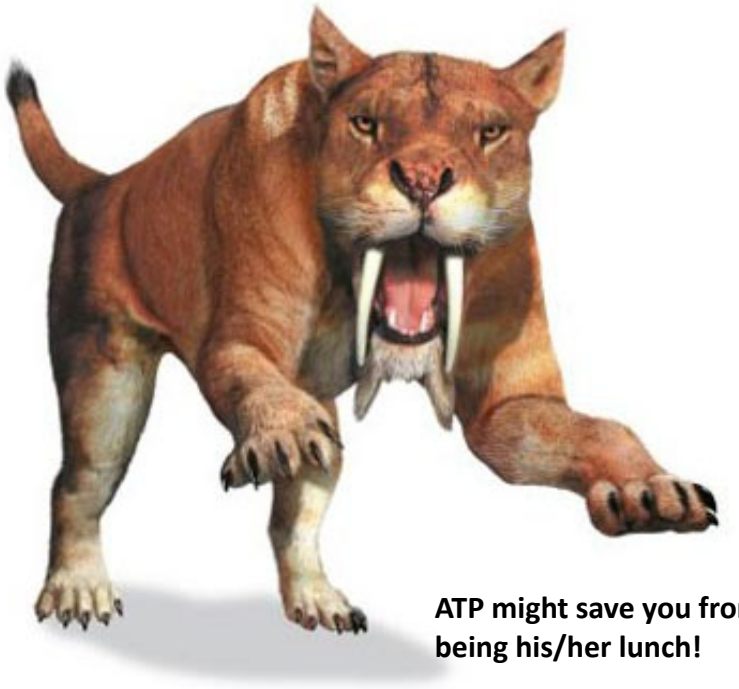

ATP might save you from being his/her lunch!

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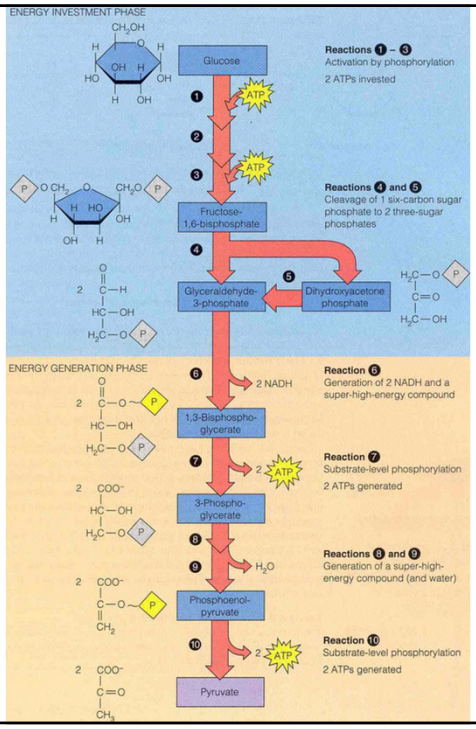

The pathway of glycolysis

Inefficient, but high capacity way of making ATP

Pyruvate feeds into the Krebs cycle (oxidative metabolism)

Pyruvate is also converted to lactate (high stress like running a marathon, or in low oxygen)

biochemstarr.wordpress.com

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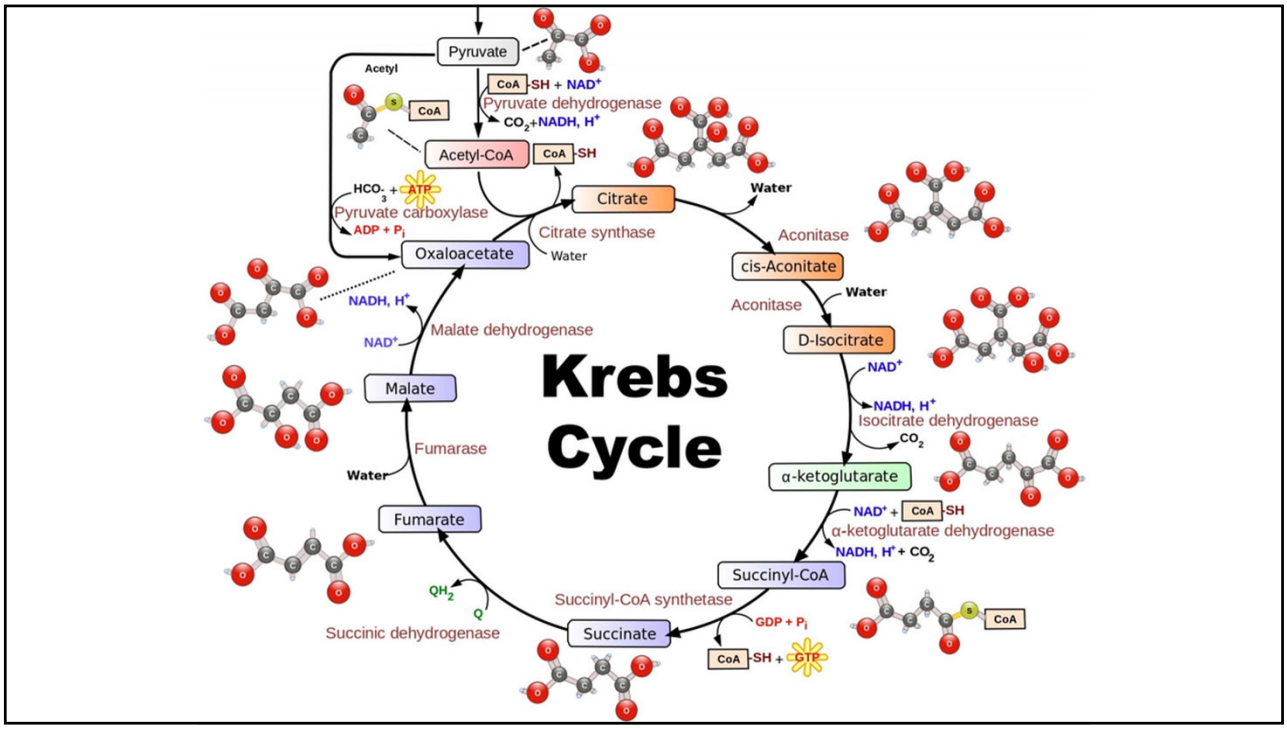

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Sir Hans Krebs

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

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(Sir) Hans Krebs

- *There was a young lady from Hyde*
- *Who ate a green apple and died*
- *Inside the lamented, the apple fermented*
- *And made cider inside her inside*

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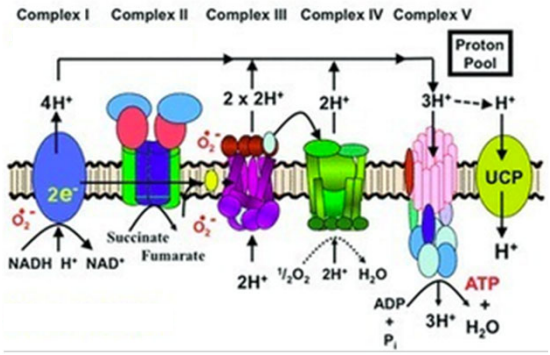
Mitochondrial oxidative phosphorylation



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.

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ATP synthetase

https://www.youtube.com/watch?v=CSrtewCJbpg

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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 (μM or mM) (acetyl CoA, ATP, S-AdMet, αKG) are regulators of epigenetics**
  - **Bile acids (μM) are the natural ligands of FXR and LXR**
  - **Other metabolites (pM or nM) may be exquisite physiological regulators of kidney function (prostaglandins, F_2-isoprostanes)**
- **Studying the metabolome requires multiple levels of science from the analytical to the physiologic to the computational**

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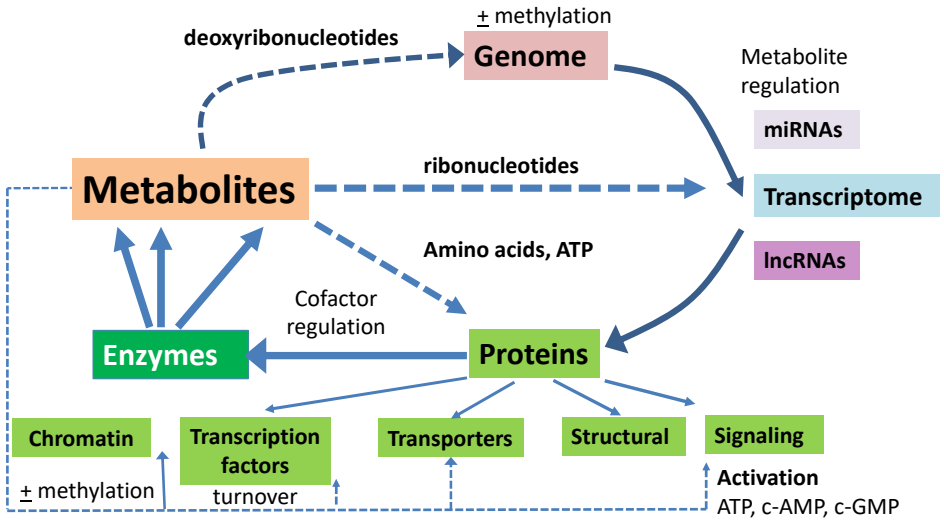
Metabolites are associated with every aspect of cellular events



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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

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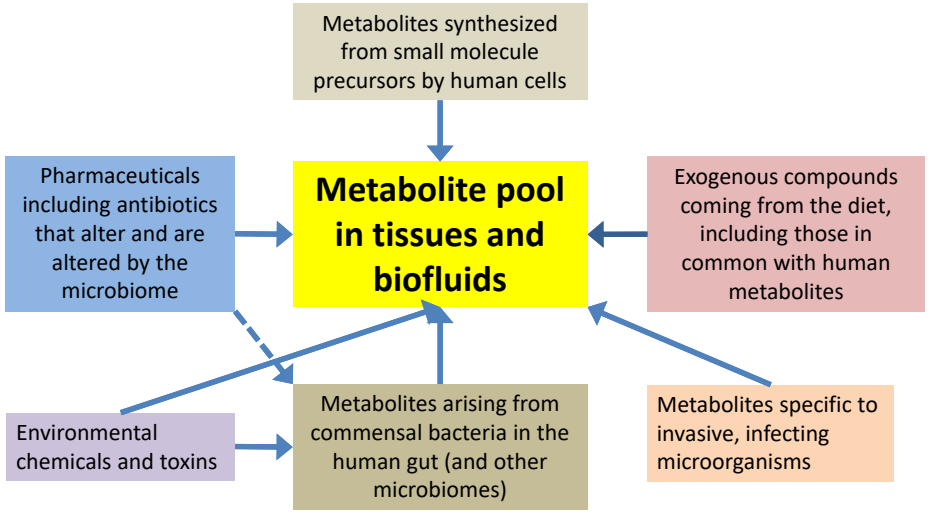
The metabolome is more than what's in textbooks



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Meat eater

Berry eater

Amino acids

Essential	Non-essential
Arg*	Ala
His	Asn
Ile	Asp
Leu	Cys
Lys	Gln
Met	Glu
Phe	Gly
Thr	Pro
Trp	Ser
Val	Tyr

Have to eat foods rich in these

Vegetarian

Fruitarian

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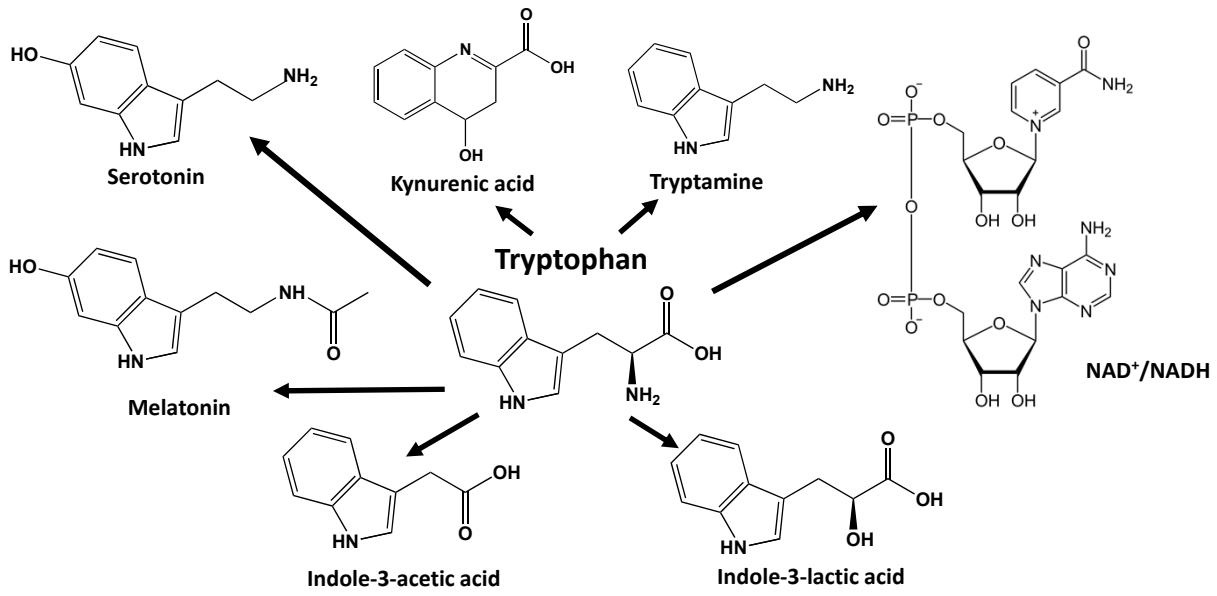
Amino acids are not just used for making proteins



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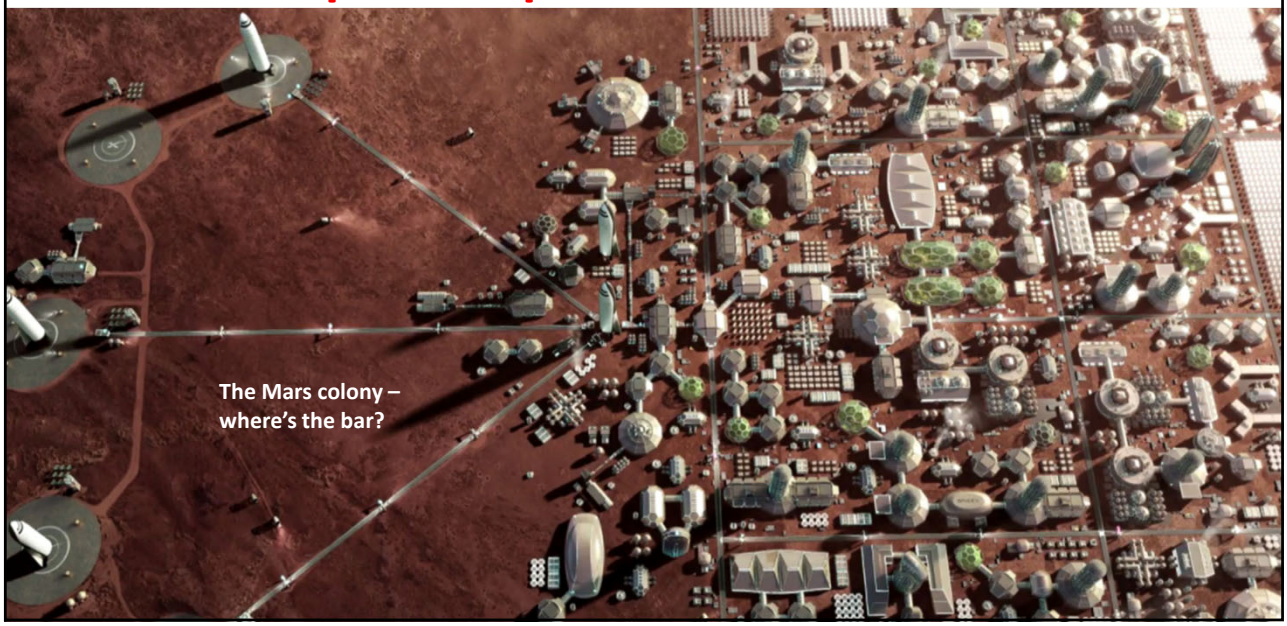
Important points to understand



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Dark beer – B vitamins

Rich in polyphenols

Yoghurt +polyphenols

Tempeh - soy

Kimchi - cabbage

Bitter beer – male hop UK

Alcohol/calories

Plain, but fermented

Ogi + hibiscus flower syrup

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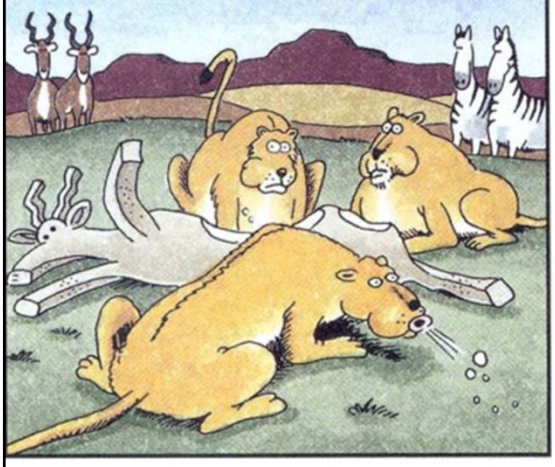
A sign you've made it when the On the Wild Side ridicules you



In sudden disgust, the three lionesses realized they had killed a tofudebeest—one of the Serengeti's obnoxious health antelopes.

Be kind to your "cat"

Vet. Pathol. 25:48–57 (1988)

Veno-occlusive Disease of the Liver in Captive Cheetah

The main hepatic lesion was seen in 60% of the sexually mature cheetah (out of 126 captive animals). Observed in 1 year olds, but got worse with age and led to liver failure. Came from supplementation of the horsemeat diet with soy protein and the phytoestrogens therein.

Cats are exquisitely sensitive to aspirin and tylenol

- **The defect is in UGT1A6 which has become a pseudogene – the WT form glucuronidates phenols (a mechanism to excrete them)**
  - **Cats are hypercarnivores**
    - **Not exposed to modern drugs or plants in which there are substantial amounts of phenols**
  - **Victims of "Use it or lose it"**
  - **Diet-driven evolution**
- **Mutations in exon 1**
  - **Stop codons at bp 274-276 and 379-381 (>10 MYA)**
- **UGT1A1 that glucuronidates bilirubin is unaffected**

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Overview of metabolome chemistry

Metabolites encompass an enormous range of chemistries

- **Gaseous**
  - **H_2, H_2S**
- **Volatile**
  - **Butyric acid, acetone, skatole**
- **Hydrophilic (water-loving)**
  - **Glucose**
- **Charged-positive/negative**
  - **Amino acids, nucleotides, organic acids, amines**
- **Hydrophobic (fat-loving)**
  - **Lipids, steroids, hydrocarbons**

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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 flavin monooxygenase (FMO3)-deficient subjects**

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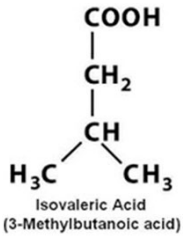
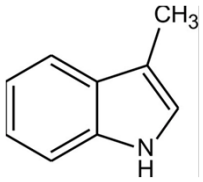
Gases and volatiles

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



Isovaleric Acid
(3-Methylbutanoic acid)

- **Flatulance**
  - **Mostly gases (H_2, CO_2 and H_2S), but with volatiles produced by colonic bacteria (skatole, from the amino acid tryptophan)**



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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**
- **React *in situ* to form a non-volatile derivative before evaporating**

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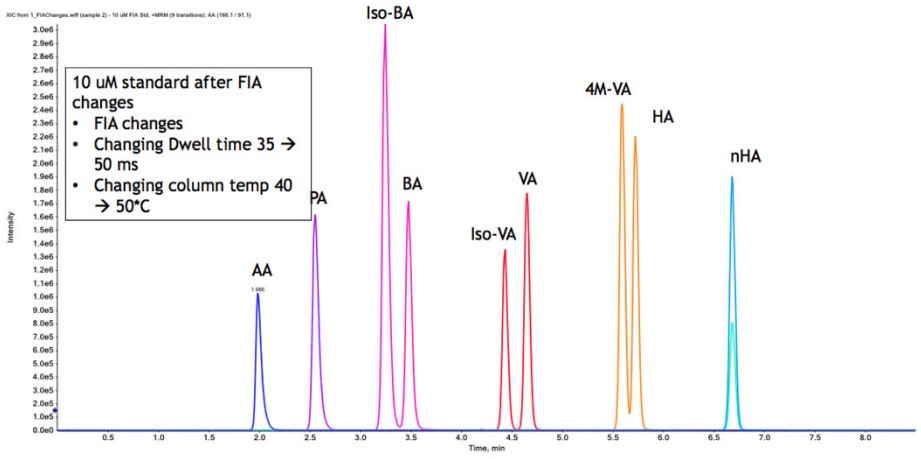
o-benzylhydroxylamine derivatives



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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**

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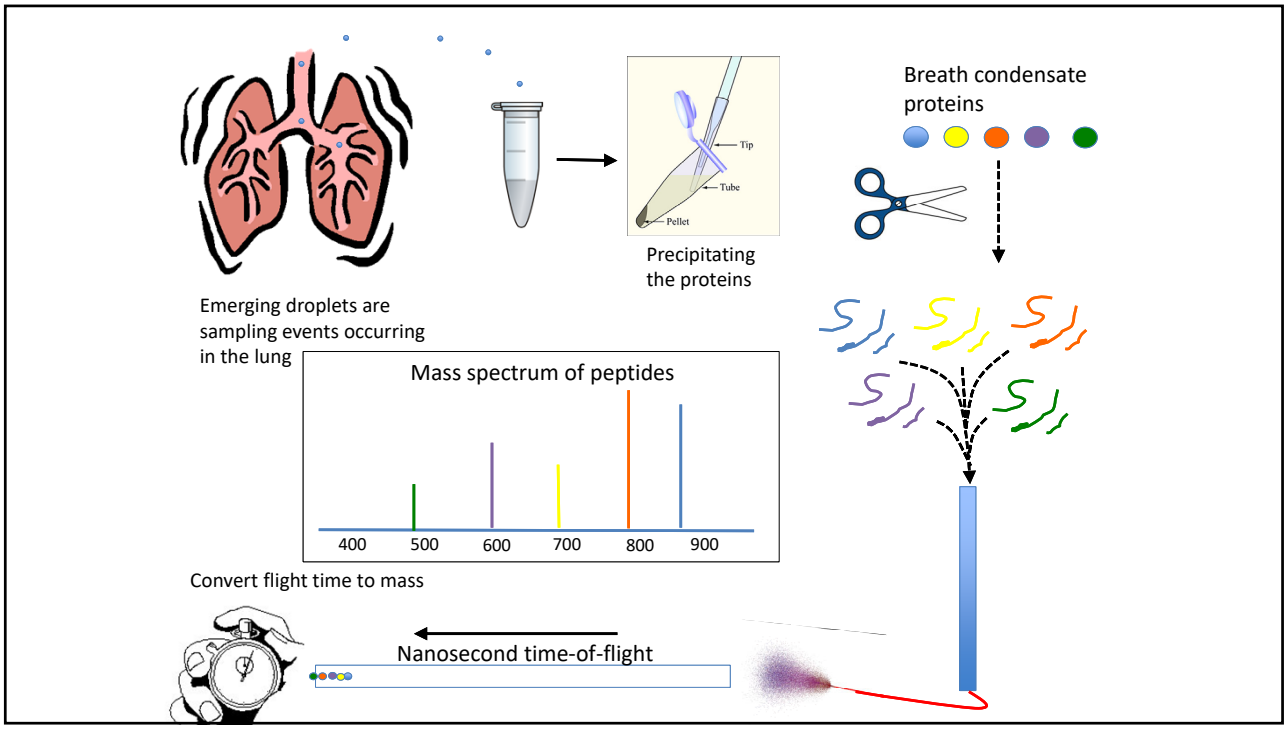

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Certain metabolites are peptides

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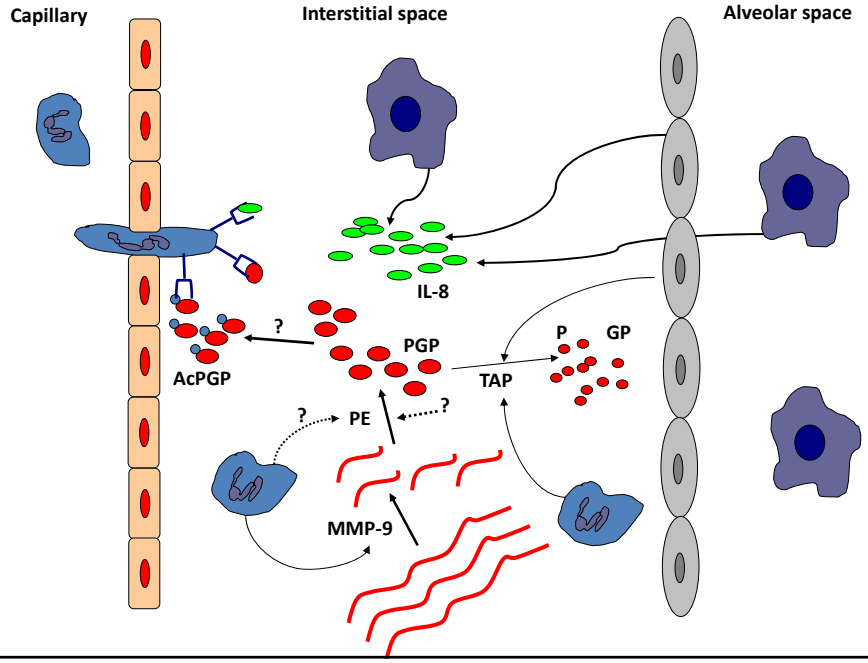
A homeostatic circuit controlling neutrophil recruitment



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PGP is a common peptide in human collagen

MFSFVDLRLLLLLAATALLTHGQEEGQVEGQDEDIPPITCVQNGLRYHDRDVWKPEPCRI
CVCDNGKVLCDDVICDETKNCPGAEVPEGECCPVCPDGSESPTDQETTGVEGPKGDTGPR
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
PGPPGAVGPAGKDGEAGAQGP**PGP**AGPAGERGEQGPAGSPGFQGL**PGP**AGPPGEAGKPGE
QGVPGDLGA**PGP**SGARGERGFPGERGVQGP**PGP**AGPRGANGAPGNDGAKGDAGAPGAPGS
QGAPGLQGMPGERGAAGL**PGP**KGDRGDAGPKGADGSPGKDGVRGLTGPIGPPGPAGAPGD
KGESGPSGPAGPTGARGAPGDRGE**PGPPGP**AGFAGPPGADGQPGAKGEPGDAGAKGDAGP
PGPAGPAGP**PGP**IGNVGAPGAKGARGSAGPPGATGFPGAAGRVGP**PGP**SGNAGP**PGPPGP**
AGKEGGKGPRGETGPAGRPGEVGP**PGPPGP**AGEKGSPGADGPAGAPGT**PGP**QGIAGQRGV
VGLPGQRGERGFPGL**PGP**SGEPGKQGPSGASGERGP**PGP**MGPPGLAGPPGESGREGAPGA
EGSPGRDGSPGAKGDRGETGPAGPPGAPGAPGA**PGP**VGPAGKSGDRGETGPAGPAGPVGP
VGARGPAGPQGPRGDKGETGEQGDRGIKGHRGFSGLQGP**PGP**PGSPGEQGPSGASGPAGP
RGPPGSAGAPGKDGLNGL**PGP**IGP**PGP**RGRTGDAGPVGP**PGPPGPPGPPGP**PSAGFDFSF
LPQPPQEKAHDGGRYYRADDANVVRDRDLEVDTTLKSLSQQIENIRSPEGSRKNPARTCR
DLKMCHSDWKSGEYWIDPNQGCNLDAIKVFCNMETGETCVYPTQPSVAQKNWYISKNPKD
KRHVWFGESMTDGFQFEYGGQGSDPADVAIQLTFLRLMSTEASQNITYHCKNSVAYMDQQ
TGNLKKALLLQGSNEIEIRAEGNSRFTYSVTVDGCTSHTGAWGKTVIEYKTTKTSRLPII
DVAPLDVGAPDQEFGFDVGPVCFL

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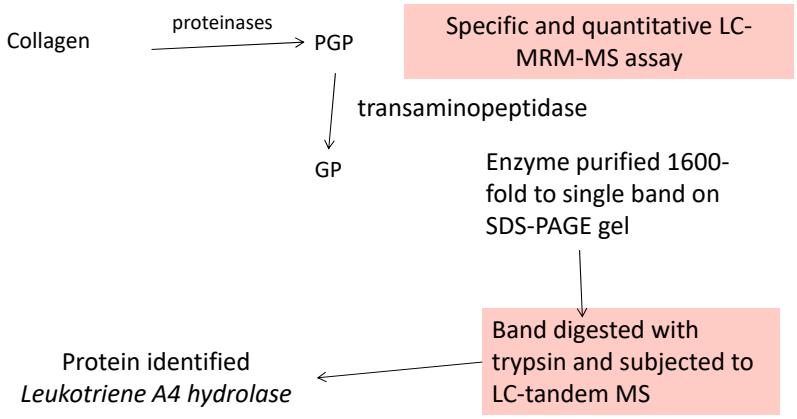
Mass spec contribution to PGP story



(Robert Snelgrove et al. Science, 2010)

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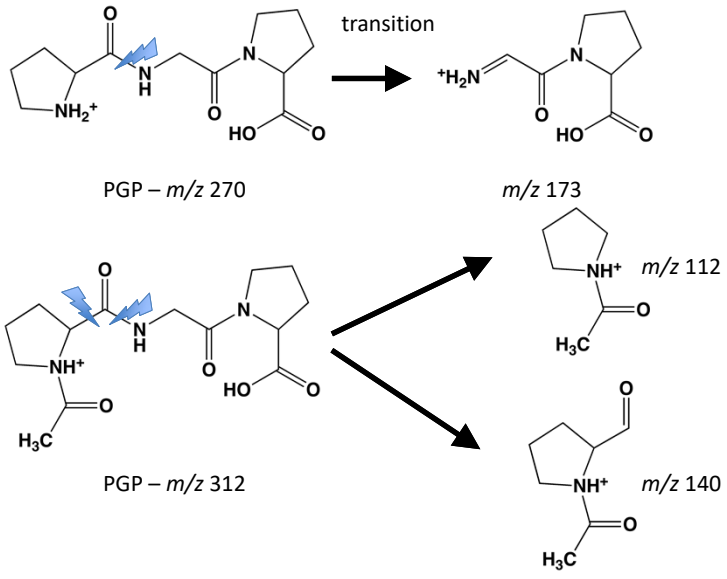
Measuring PGP and acetyl-PGP



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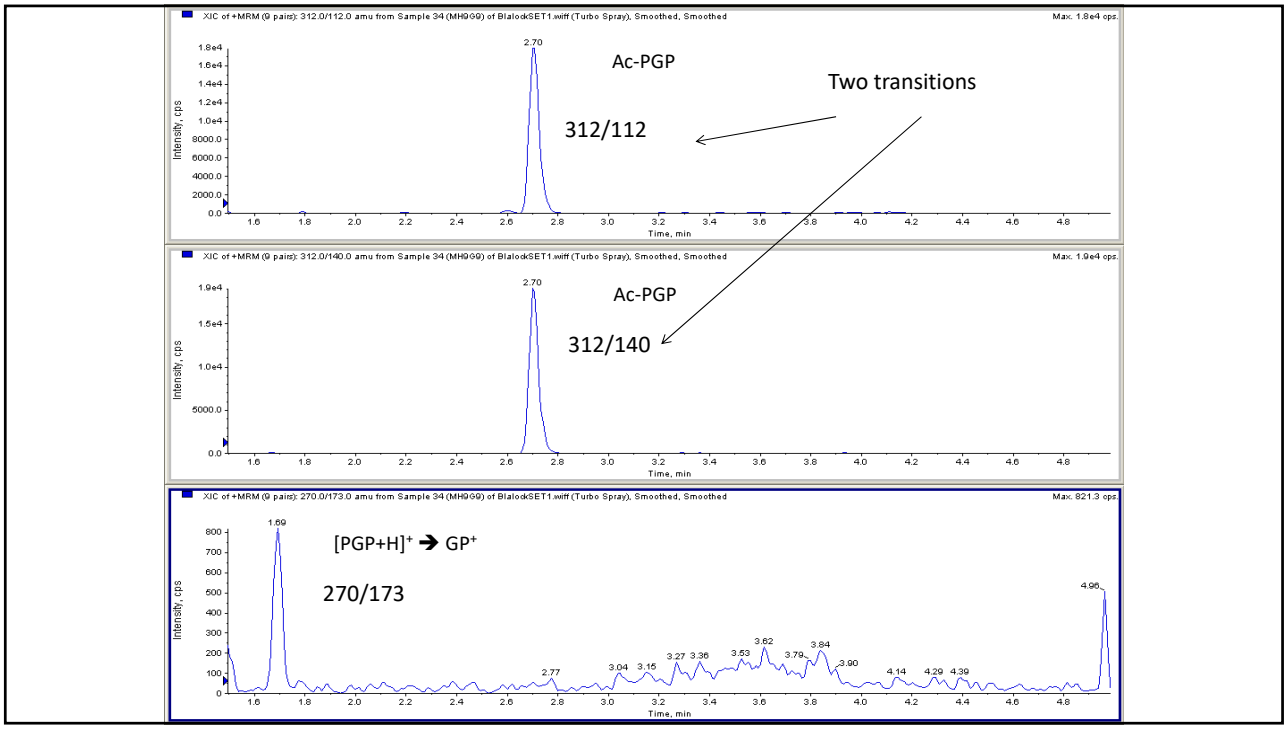

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Metabolopeptidomics or peptidometabolomics

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

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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?

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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

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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**
  - **<u>However, it is a true metabolite, i.e., it is made from smaller precursors without the direct aid of ribosomes</u>**

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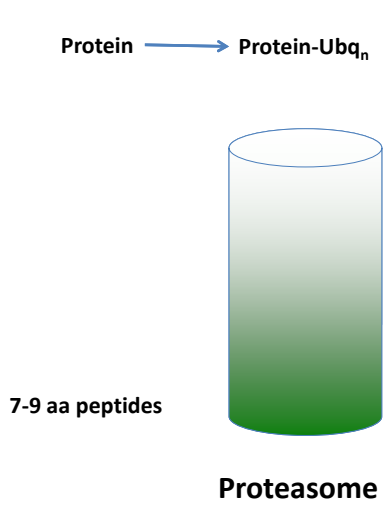
Are there other sources of short peptides?

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



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Hydrophilic metabolites

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

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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, α-ketoglutarate, Succinate, Fumarate, Malate, Oxaloactate and those resulting from pathway defects**
  - **Nucleotides**
    - **ATP, ADP, AMP, GTP, etc.**

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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)

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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)

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Hydrophobic metabolites

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

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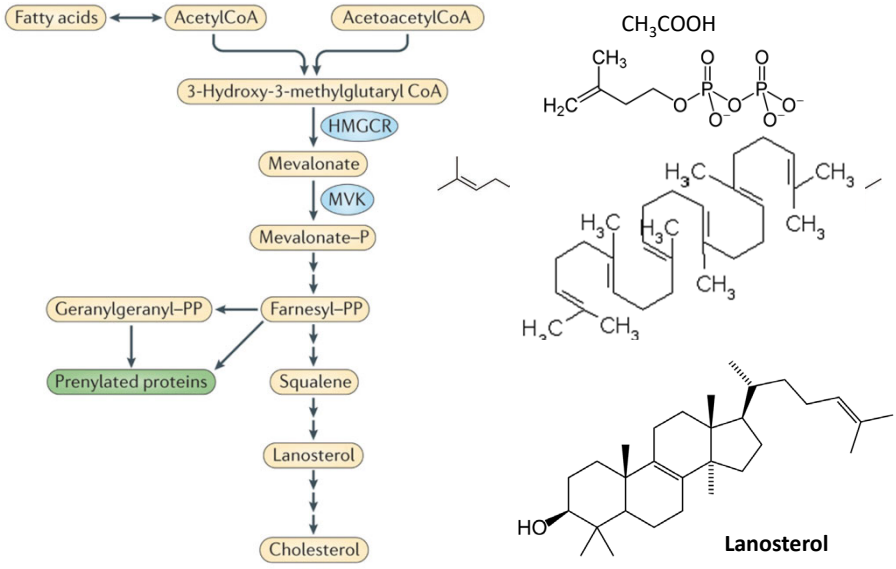
Isoprenoids and sterols



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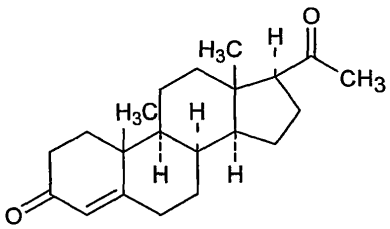
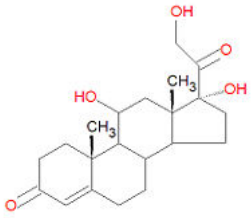
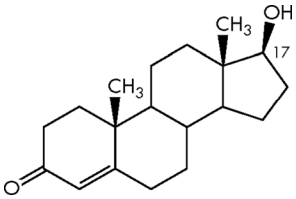
Steroids



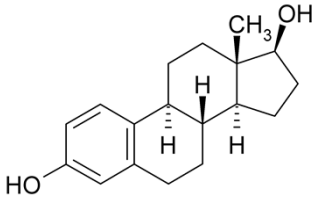
Progesterone



Cortisol



Testosterone



17β-estradiol

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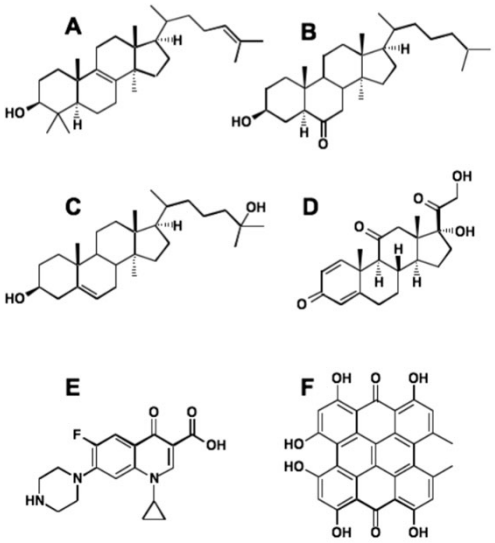
Importance of sterols and other compounds in lens cataracts



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 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.

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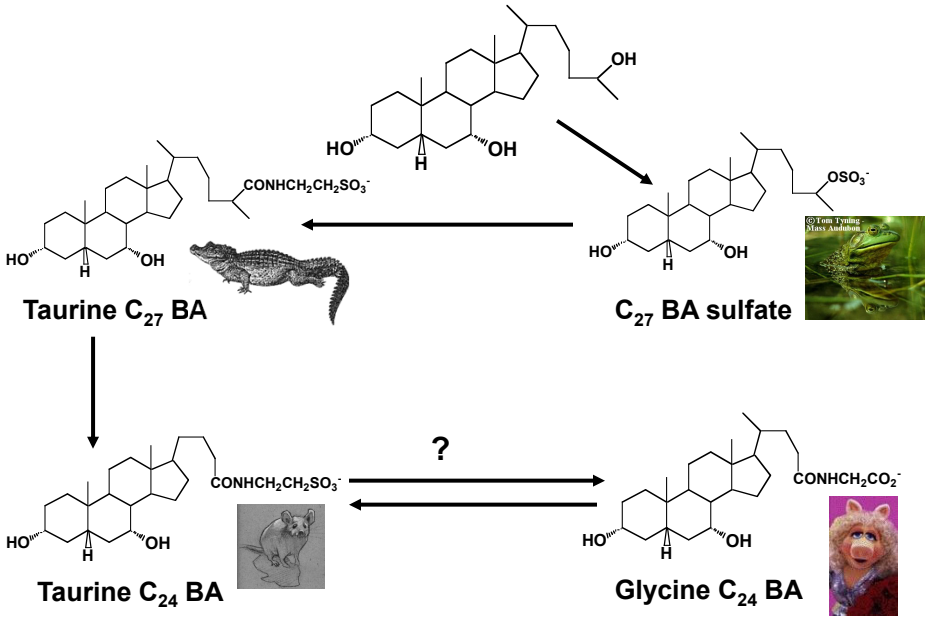
Evolution of bile acid conjugation



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The vitamins

Lack of these leads to serious illness, but not death

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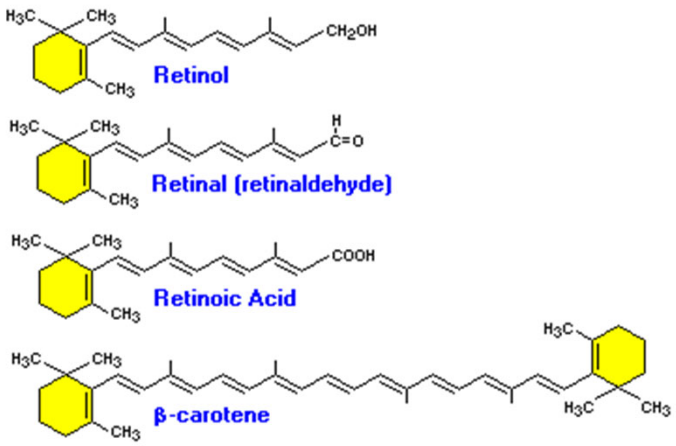
Vitamin A



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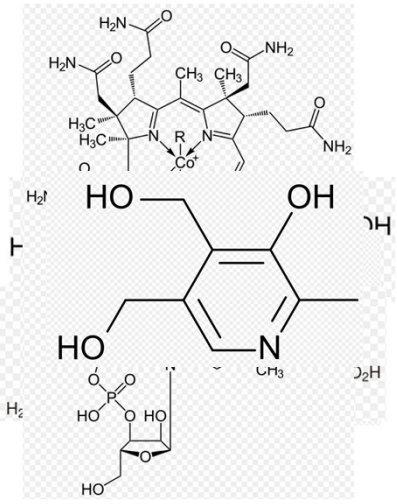
Vitamin B

- **They are all water-soluble**
  - **Vit B_1 – thiamine**
  - **Vit B_2 – riboflavin**
  - **Vit B_3 – niacin**
  - **Vit B_5 - pantothenic acid**
  - **Vit B_6 – pyridoxine**
  - **Vit B_7 – biotin**
  - **Vit B_9 – folic acid**
  - **Vit B_{12} – cobalamins**



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

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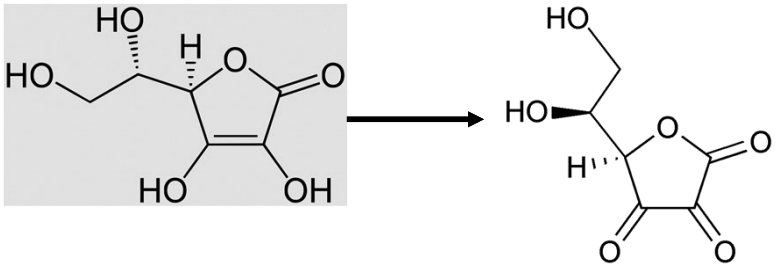
Vitamin C

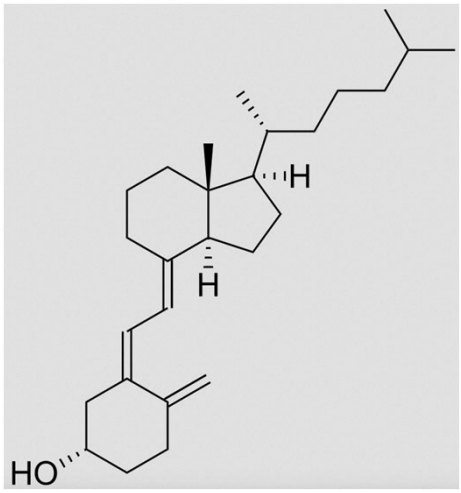
- **Ascorbic acid**



dehydroascorbic acid

53

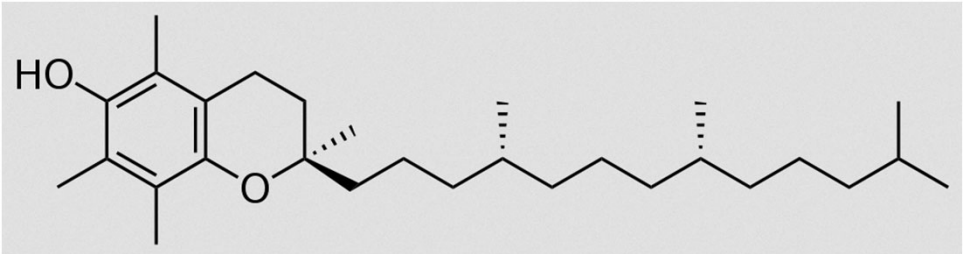
Vitamin D



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

54

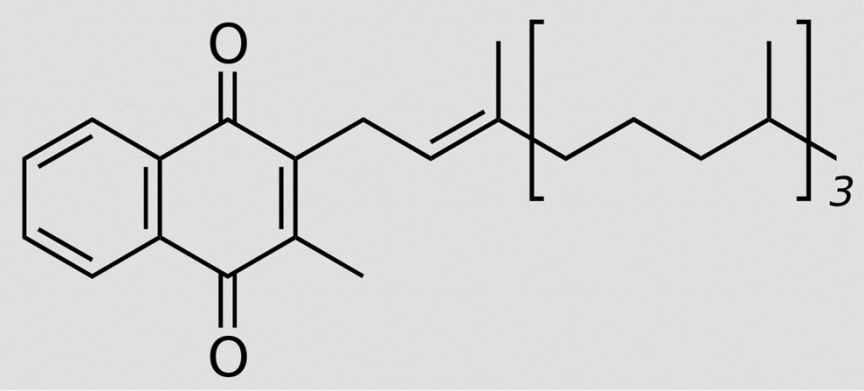
Vitamin E



Found in oils from plants

55

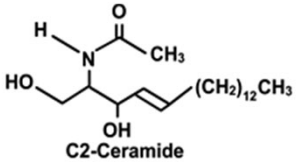
Vitamin K



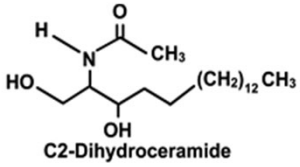
Is an anticoagulant – needed to stop bleeding

56

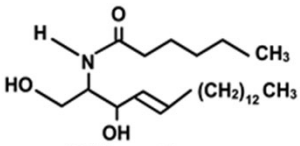
Phospholipids



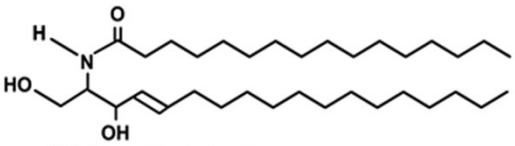
C2-Ceramide



C2-Dihydroceramide



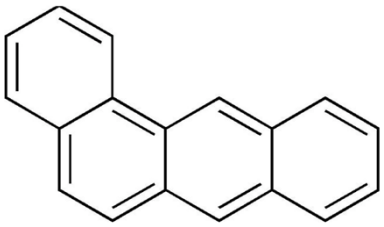
C6-Ceramide



C16-Ceramide (natural)

57

Hydrocarbons



Benz[a]anthracene
In smoke from barbecued meat

Cetyl palmitate
In hair shampoo

58

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?

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Etc., etc.

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