



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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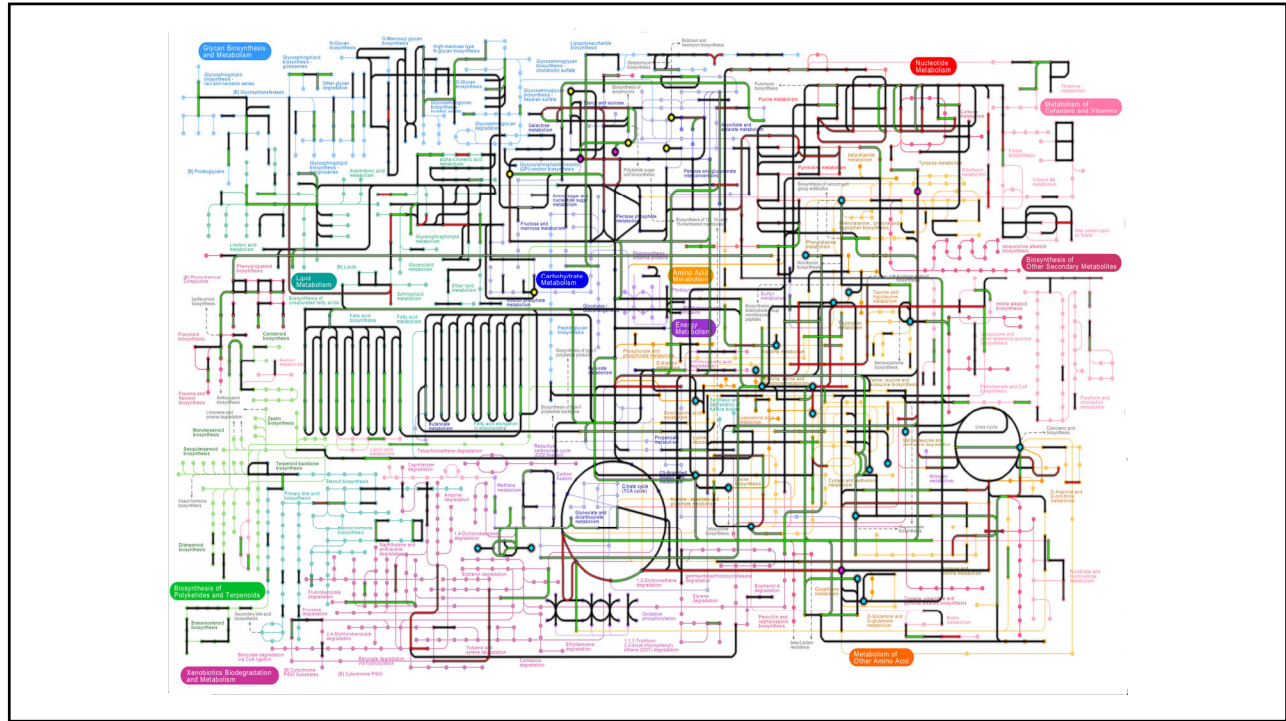
The metabolome is more than what's in textbooks

Metabolites synthesized
from small molecule
precursors by human cells



**Metabolite pool
in tissues and
biofluids**

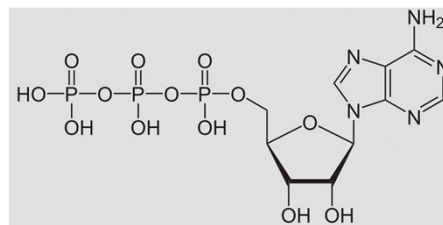
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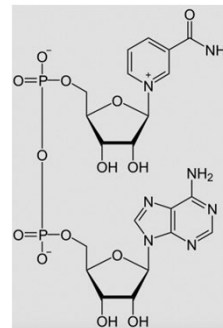
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Critical metabolites

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

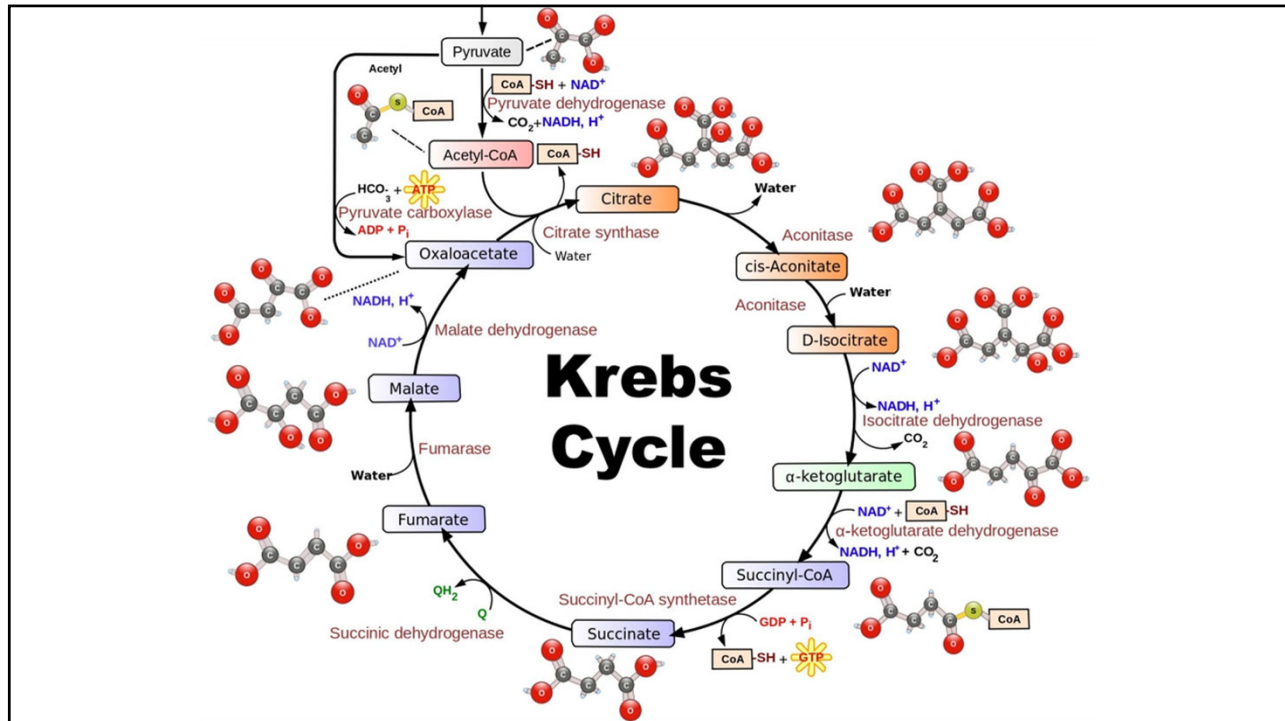
Inefficient, but high capacity way of making ATP

Pyruvate feeds into the Krebs cycle (oxidative metabolism)

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

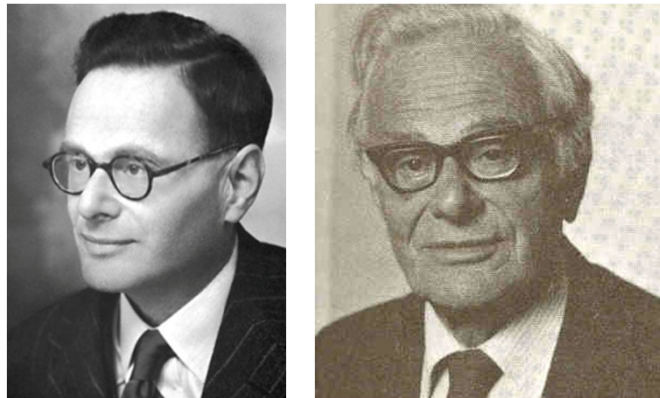
biochemstarr.wordpress.com

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Sir Hans Krebs



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

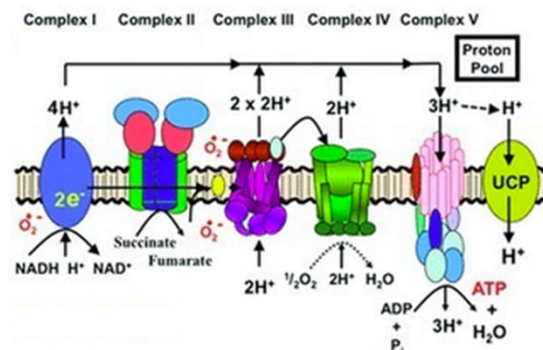
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(Sir) Hans Krebs

- *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

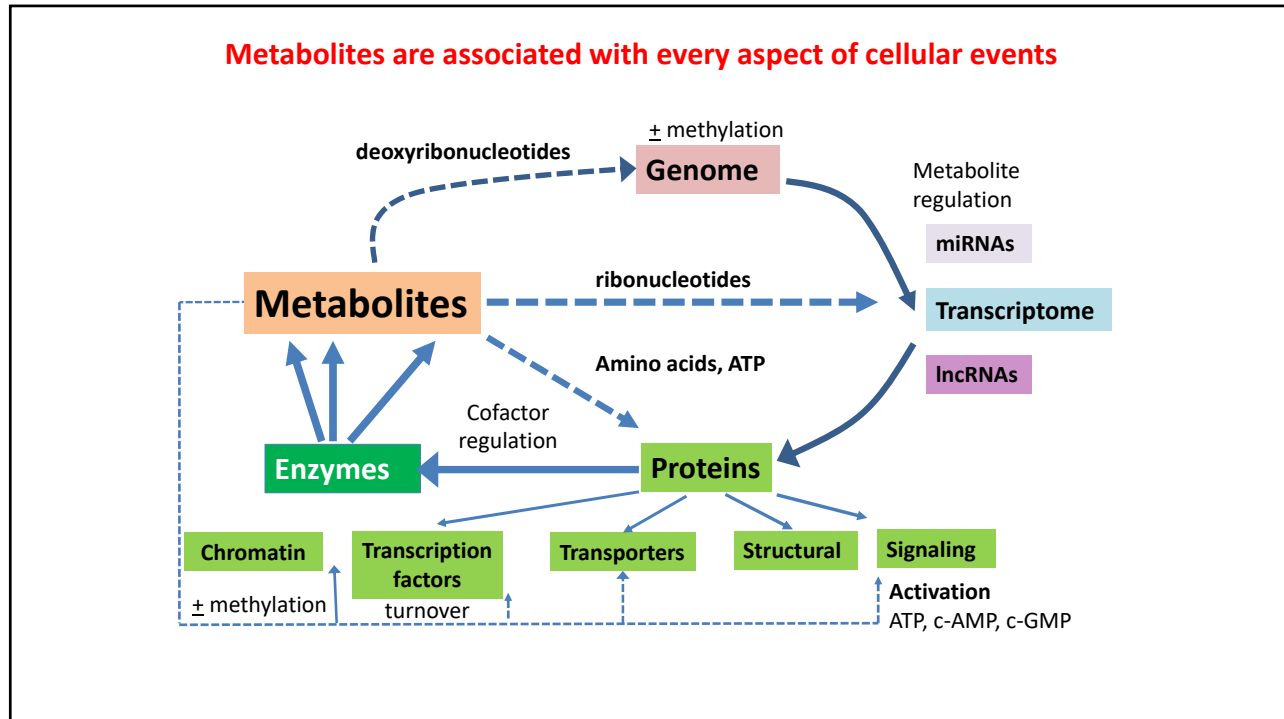
<https://www.youtube.com/watch?v=CSrtewCJbpg>

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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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Metabolism and time

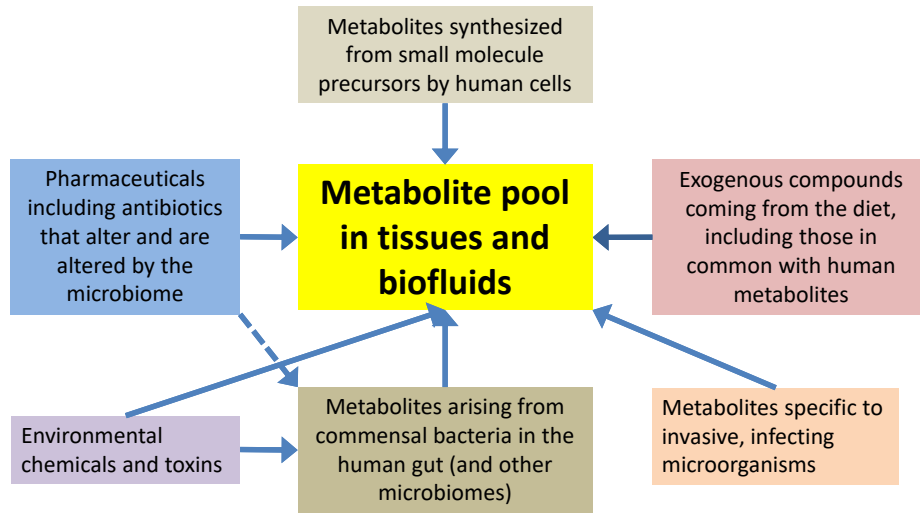
- Not only should metabolites appear in the right place, there is also the question of the importance of the timescale
- Metabolism defects in the heart may be only seconds away from death – rogue waves in metabolism??
- Irreversible damage to the brain may occur in minutes
- Go/No-Go decisions for a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

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

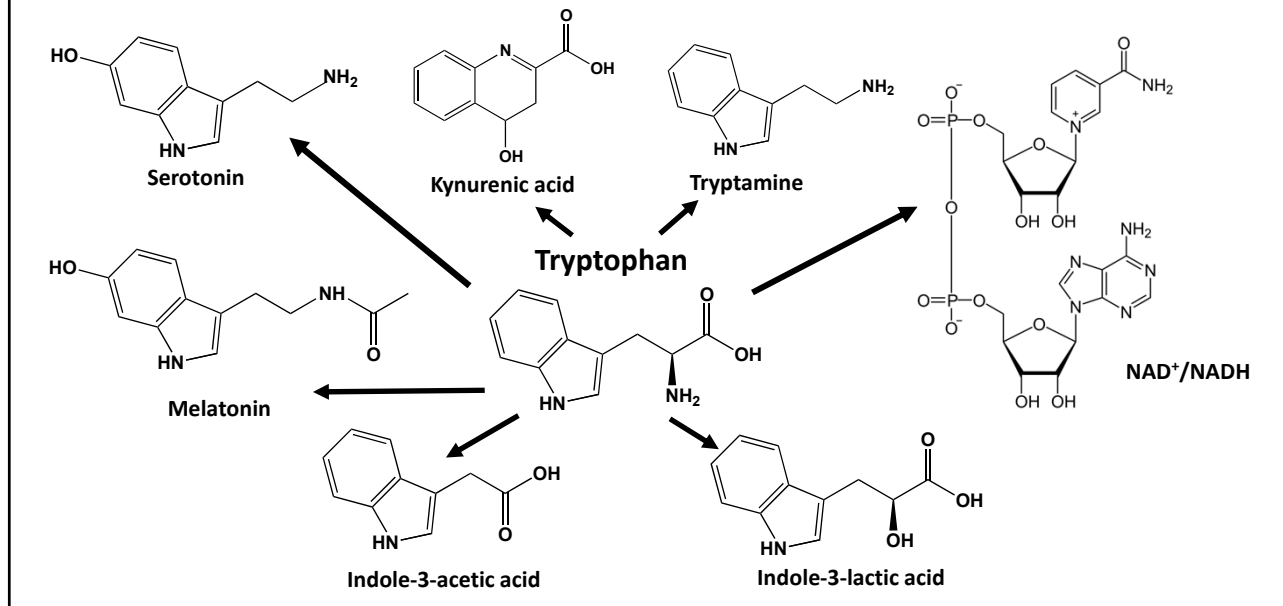
Vegetarian

Berry eater

Fruitarian

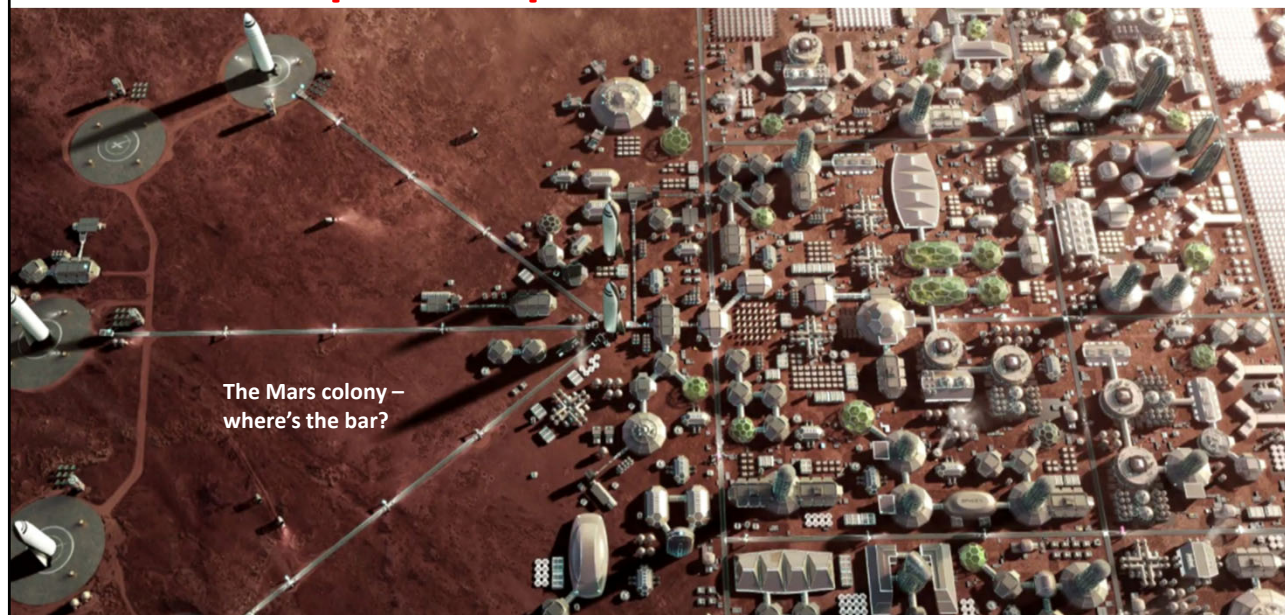
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Amino acids are not just used for making proteins

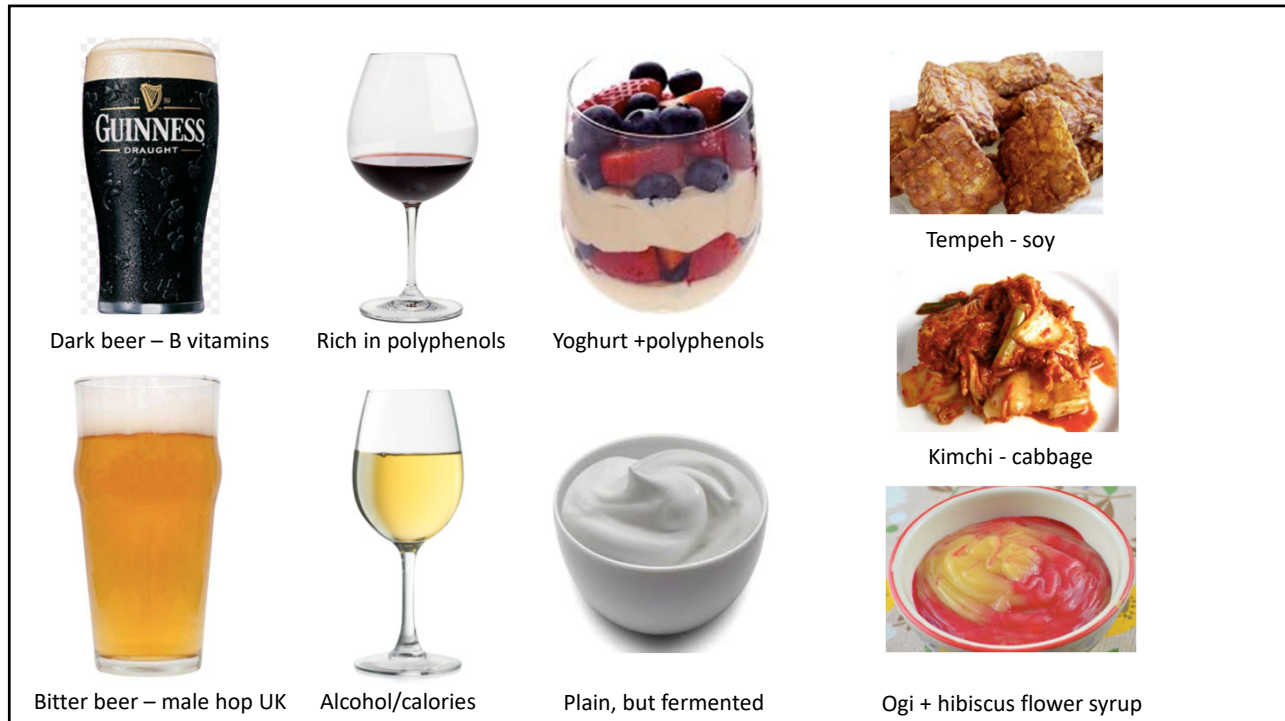


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Important points to understand



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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. 25:46-57 (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**
 - H₂, H₂S
- **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

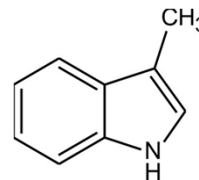
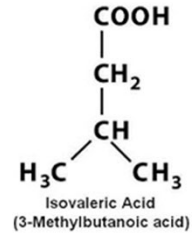
- **In breath**
 - H₂ from reductive anerobic bacteria
 - Lactose-intolerant
 - Measure of gut transit (typically 4-6 hours)
 - CO₂
 - From all carbon-containing substrates
 - From specific ¹³C-labelled substrates
 - Acetone (in diabetics)
 - Trimethylamine
 - From fish, or flavin monooxygenase (FMO3)-deficient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

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Gases and volatiles

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

- **Flatulence**
 - **Mostly gases (H₂, CO₂ and H₂S), but with volatiles produced by colonic bacteria (**skatole**, from the amino acid tryptophan)**



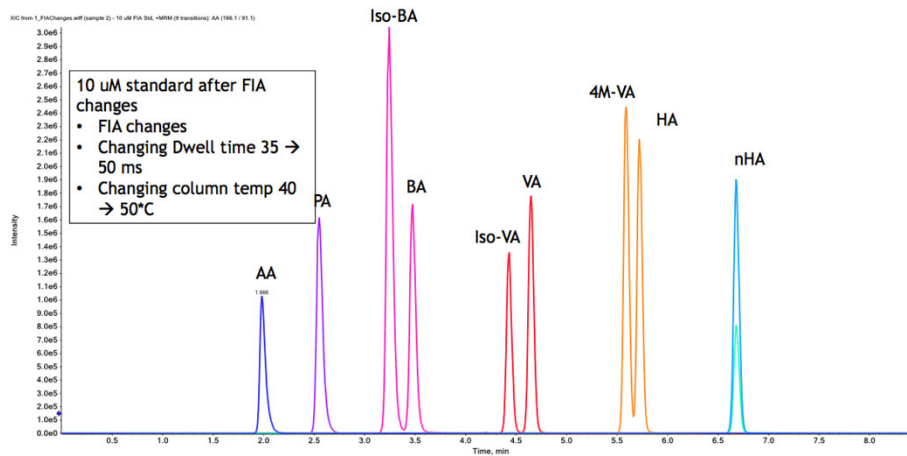
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Other volatiles

- **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

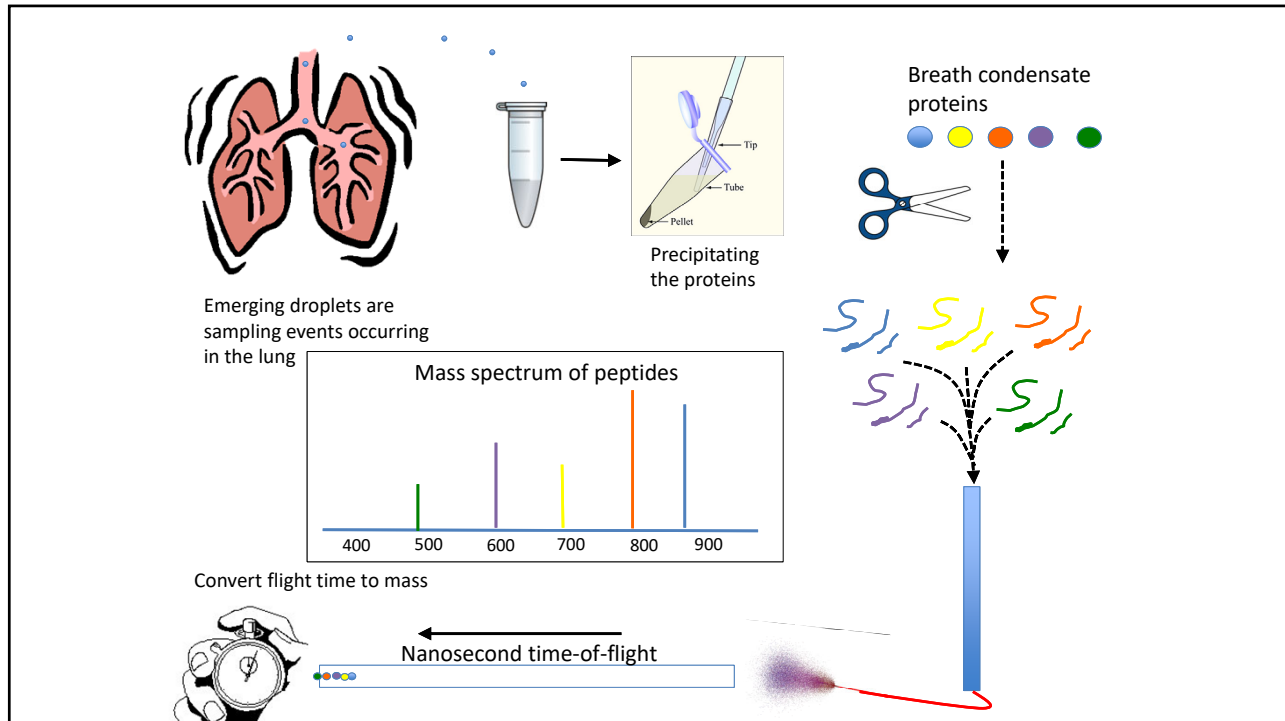


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Breath condensates

- 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

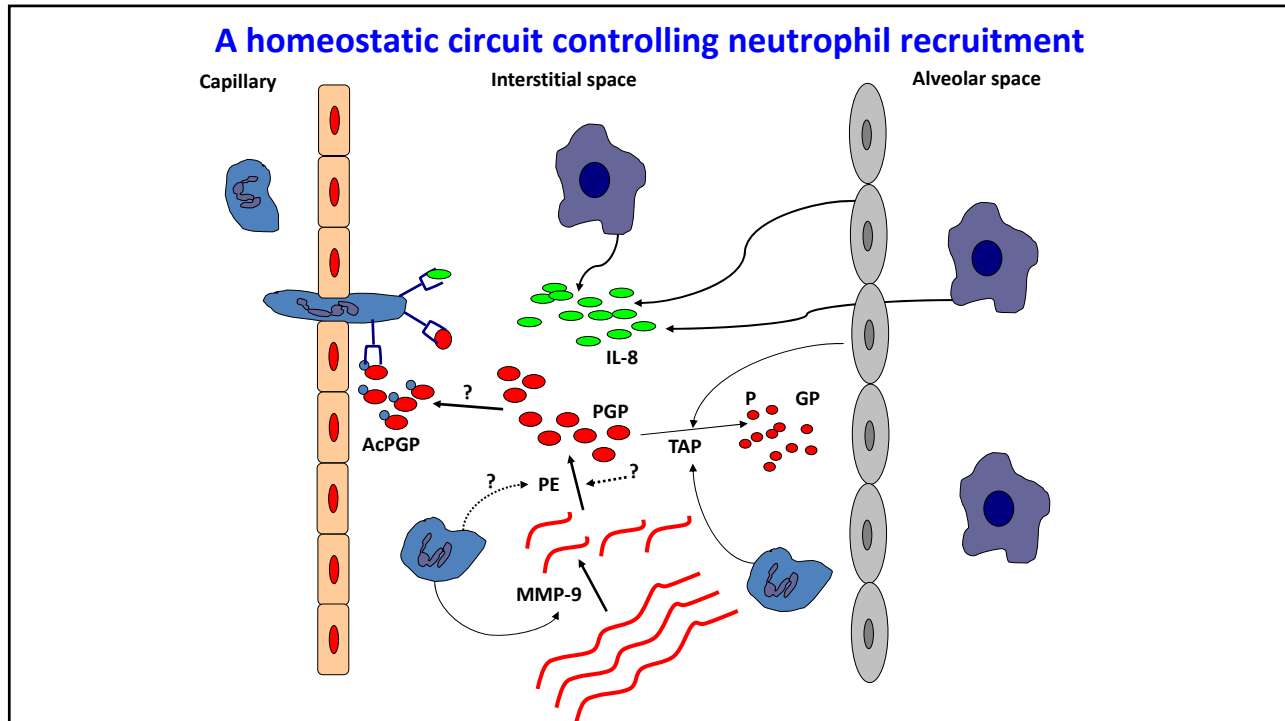
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Certain metabolites are peptides

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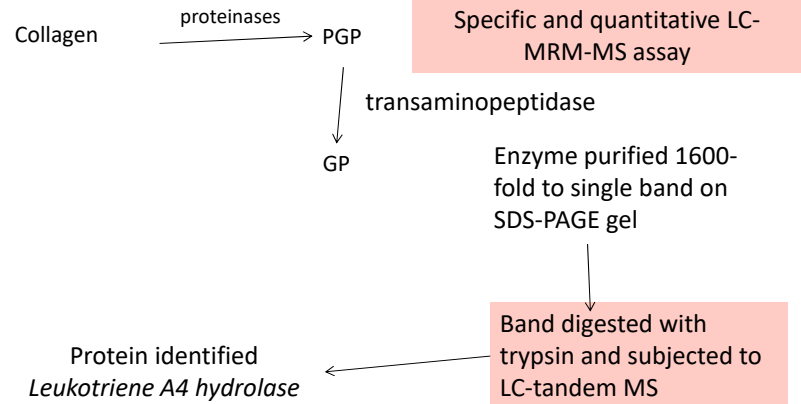
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PGP is a common peptide in human collagen

MFSFVDLRLLLLLAATALLTHGQEEGQVEGQDEDI PPIITCVQNGRLRYHDRDVWKPEPCRI
 CVCDNGKVLCDDDVICDETKNCPGAEVPEGECCPVC PDGSESP TDQETTGVVEGPKGDTGPR
 GPRGPAGPPGRDGI PGQPGLPGP **PGPPGPPGP** PGLGGNFAPQLSYGYDEKSTGGISV **PGP**
 MGPSGPRGL **PGP** PGAG **PGP** QGFQGPPEPEPGASGPMGPRGP **PGP** PGKNGDDGEAGKPR
 PGERGP **PGP** QGARGLPGTAGLPGMKGHRGFSGLDGAKGDAGPAGPKGEPGSPGENGAPGQ
 MPRGLPGERGRPGAG **PGP** AGARGNDGATGAAGP **PGP** TGPAGPPGFPFPAVGAKEAGPQGP
 RGSEGPQGV RGE **PGPPGP** AGAAGPAGNPGADGQPGA KANGAPGIAGAPFPGARGPSG
 QGPGGP **PGP** KGNSGEPGAPGSKGDTGAKGE **PGP** VGVQGP **PGP** AGEEGKR GARGE **PGP** TGL
PGP PGERGGPGSRGFPGADGVAGPKGPAGERGS **PGP** AGPKGSPGEAGRPEAGLPKAKGL
 TGSPGS **PGP** DGKTGP **PGP** AGQDGR **PGPPGP** PGARGQAGVMGF **PGP** KGAAGEPGKAGERV
PGP PGAVGPAGKDG EAGAQQGP **PGP** AGPAGERGEQGPAGSPGFQGL **PGP** AGPPGEAGKPGE
 QGVGPD LGA **PGP** SGARGERGF PGERGVQGP **PGP** AGPRGANGAPNGDAGKGDAGAPGAPGS
 QGAPLQGM PGERGAAGL **PGP** KGDRGDAGPKGADGSPKDGVRGLTGP IGP PGAPAGPD
 KGESGSPGAPGTGARGAPDRGE **PGPPGP** AGFAGPPGADGQPGA KGE PGDAGAKGDAGP
PGP AGPAGP **PGP** IGNV GAPGAKGARGSAGPPGATGFPGAAGR VGP **PGPSGNAGP** **PGPPGP**
 AGKEGGKPRGETGPAGRPGEVGP **PGPPGP** AGEKGS PGADGPAGAPGT **PGP** QGIAGQRGV
 VGLPGQRGERGFPL **PGP** SGEPGKQGPSGASGERGF **PGP** MGPPLAGPPGESGREGAPGA
 EGSPGRDGS PGAKDRGETGPAGPPGAPGAPGA **PGP** VGPAGKSGDRGETGPAGPAGPVGP
 VGARGPAGPQGRGDKGETGEQDRGIKGRGFSGLQGP **PGP** PGSPGEQGPSGASGPAGP
 RGPPGSAGAPGKDGLNGL **PGP** IGP **PGPR** RRTGDAGPVGP **PGPPGP** **PGPPGP** PSAGFDFS
 LPQPQEKAHHDGGRYRADDANVVRDRDLEVDTTLKSLSQQIENIRSPGSRKNPARTCR
 DLKMHSDWKSGEYWIDPNQGCNLDAIKVFCNMETGETCVYPTQPSVAQKNWYISKNPKD
 KRHVWFGESMTDGFQFEYGGQSDPADVAIQTLFLRLMSTEASQNIITYHCKNSVAYMDQQ
 TGNLKKALLLQGSNEIEIRAEGNSRFTYSVTVDGCTSHTGAWGKTVIEYKTTKTSRLPII
 DVAPLDV GADPQEF GFDVGPVCF L

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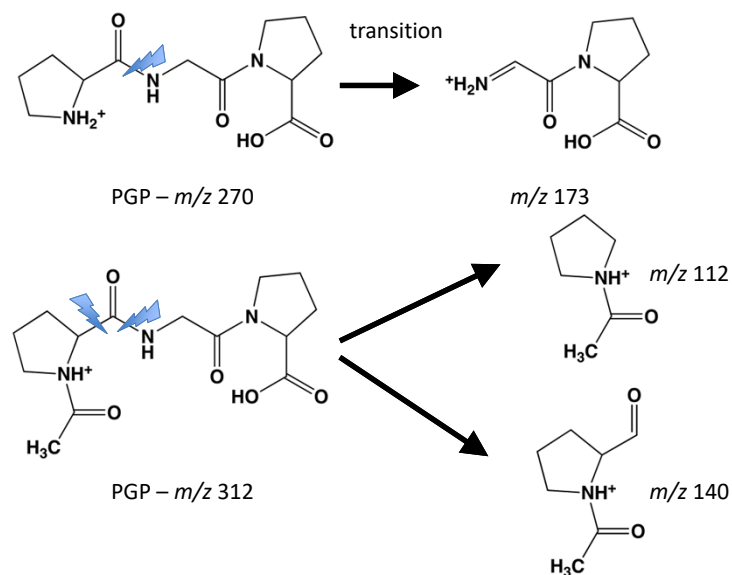
Mass spec contribution to PGP story



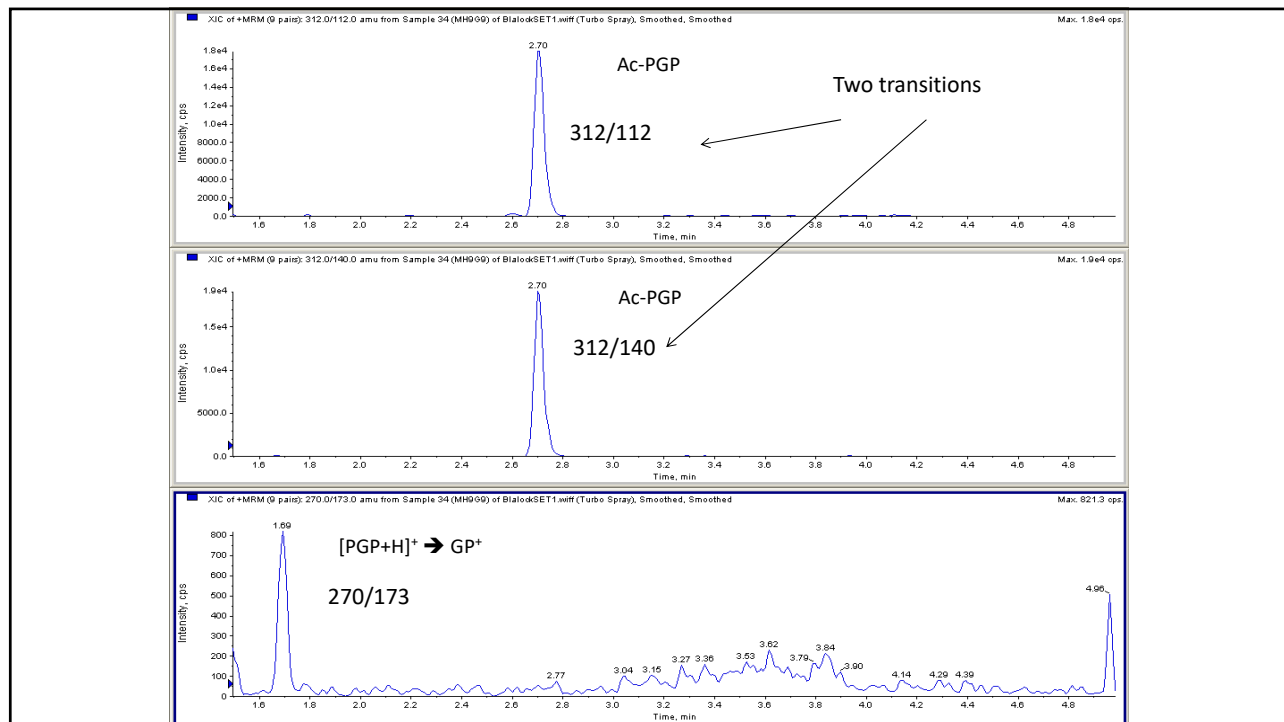
(Robert Snelgrove et al. Science, 2010)

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Measuring PGP and acetyl-PGP



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Metabolo-peptidomics 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**
 - However, it is a true metabolite, i.e., it is made from smaller precursors without the direct aid of ribosomes

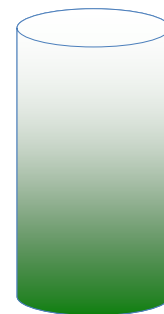
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Are there other sources of short peptides?

- 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

Protein → Protein-Ubq_n

7-9 aa peptides



Proteasome

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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)

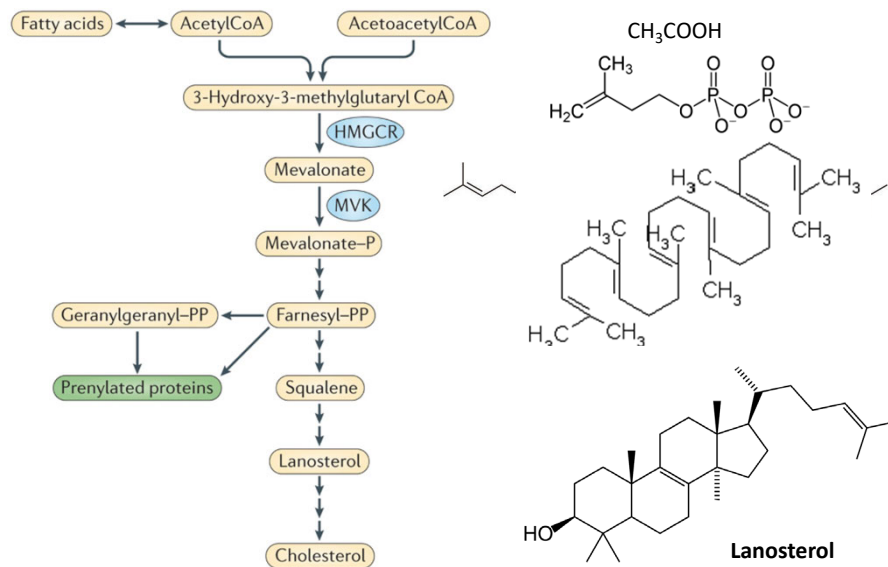
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Hydrophobic metabolites

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

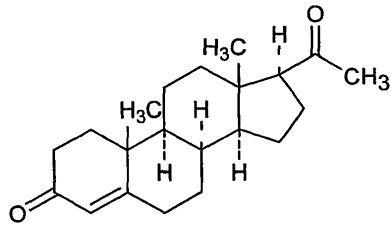
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Isoprenoids and sterols

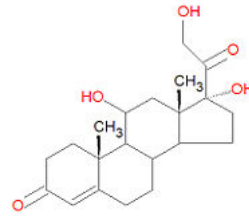


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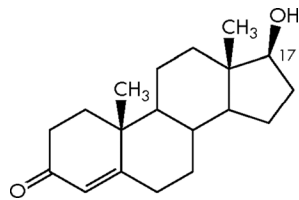
Steroids



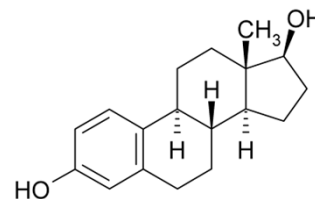
Progesterone



Cortisol



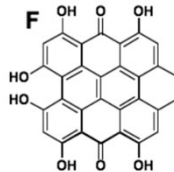
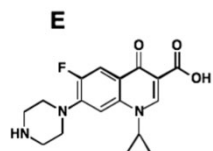
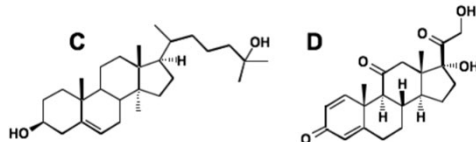
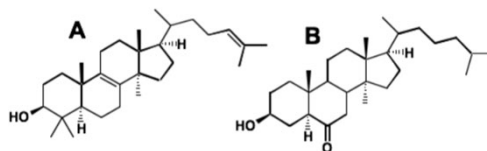
Testosterone



17β-estradiol

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Importance of sterols and other compounds in lens cataracts

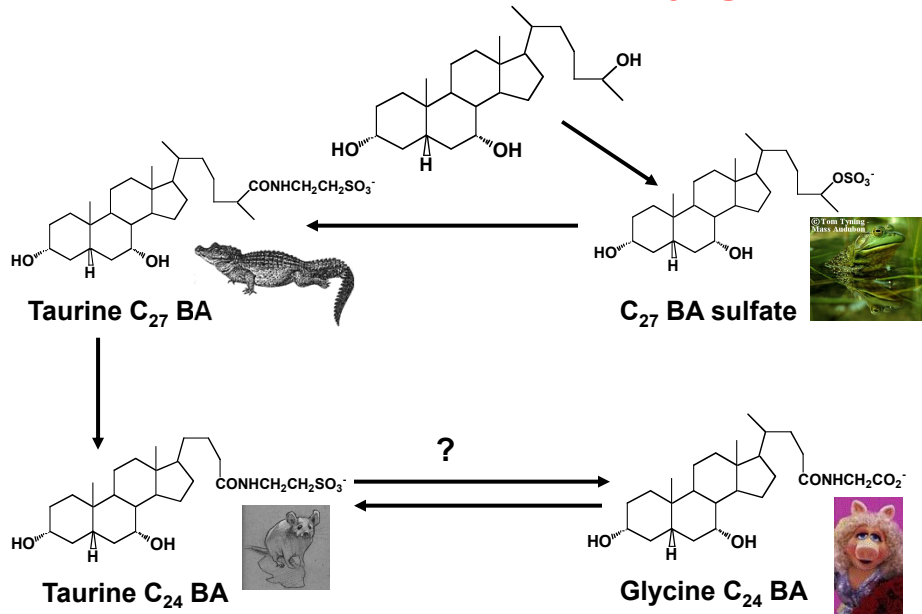


Structures A, B and C (all sterols) have recently been shown to have the property of “dissolving” lens cataracts. Cholesterol, on the other hand, has no effect. Other sterols observed in *cerebrotendinous xanthomatosis* promote cataracts.

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.

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Evolution of bile acid conjugation



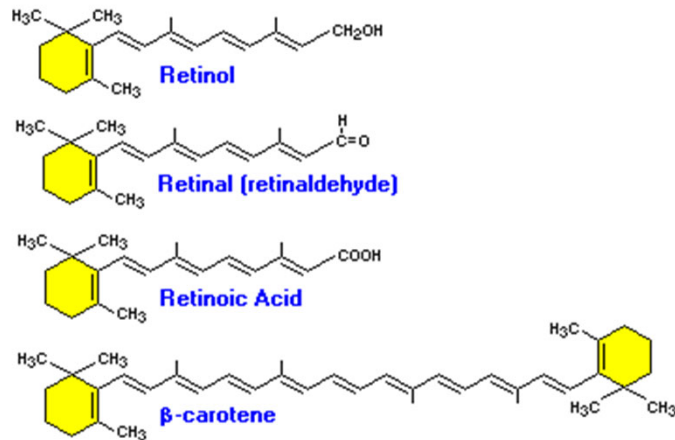
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The vitamins

Lack of these leads to serious illness, but not death

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Vitamin A

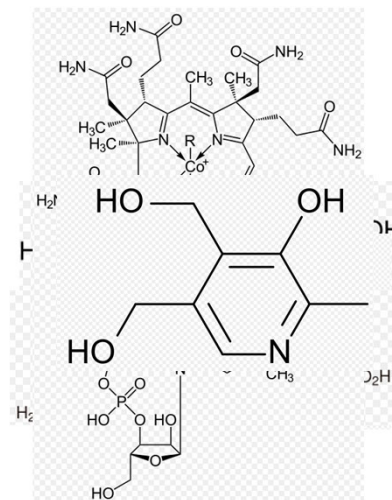


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Vitamin B

- They are all water-soluble

- Vit B₁ – thiamine
- Vit B₂ – riboflavin
- Vit B₃ – niacin
- Vit B₅ - pantothenic acid
- Vit B₆ – pyridoxine
- Vit B₇ – biotin
- Vit B₉ – folic acid
- Vit B₁₂ – cobalamins

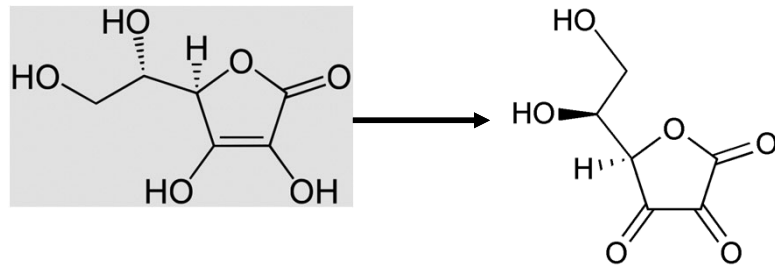


They are not made by human enzymes and if deficient in the diet cause disease

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Vitamin C

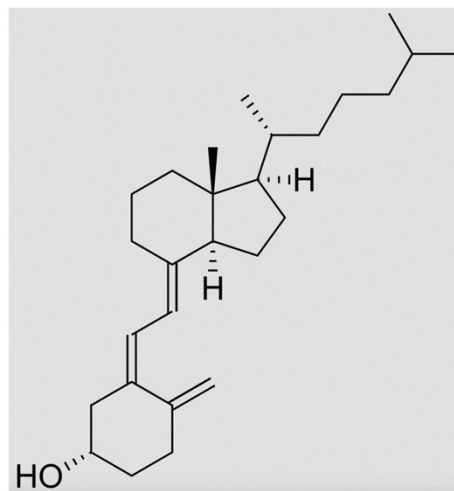
- Ascorbic acid



dehydroascorbic acid

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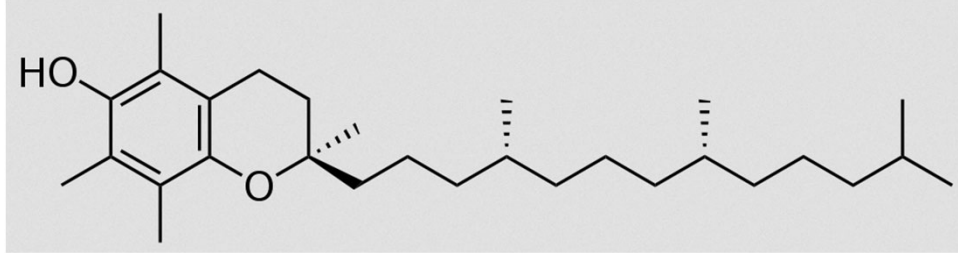
Vitamin D



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

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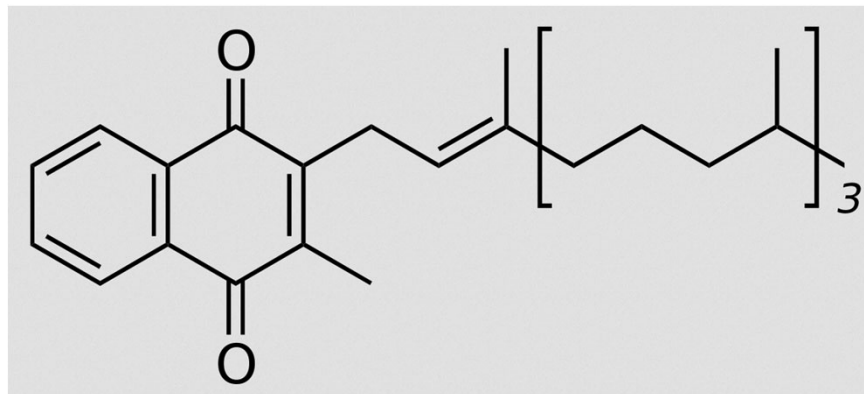
Vitamin E



Found in oils from plants

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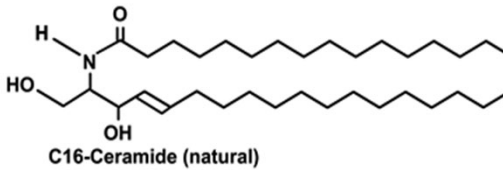
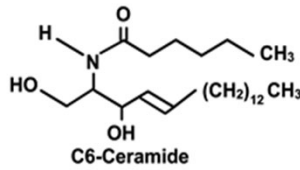
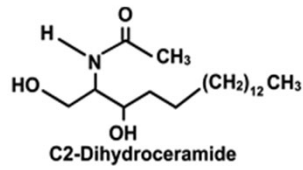
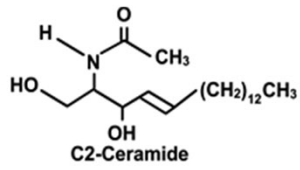
Vitamin K



Is an anticoagulant – needed to stop bleeding

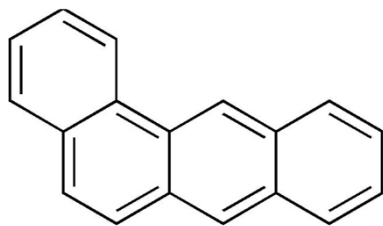
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Phospholipids



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Hydrocarbons



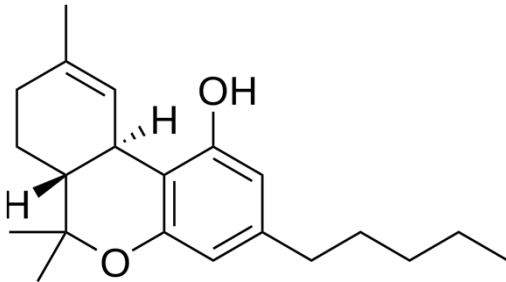
Benz[a]anthracene
In smoke from barbecued meat



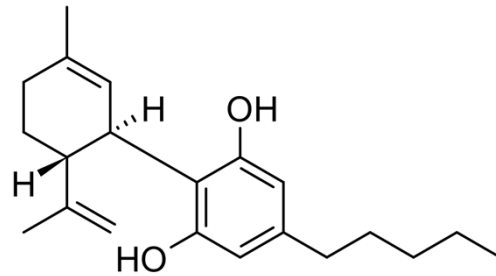
Cetyl palmitate
In hair shampoo

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Cannabinoids



Tetrahydrocannabinol (THC)



Cannabidiol, ring-opened version of THC

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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.