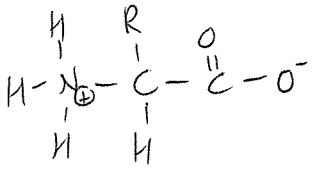
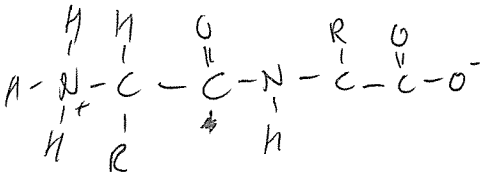
Amino Acids – contain amino group, carboxylate group

2 joined amino acid = dipeptide

↳ connected by peptide bond



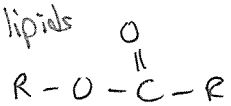


alcohol → (oxidised) aldehyde or ketone → (oxidised) carboxylic acid → CO_2

carbonyl, name depends on where R-OH is located.

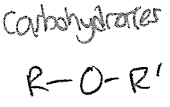
Common linking in biomolecules: the chemical properties of these linkages contribute to the structure (shape) and the reactivity of biopolymers [proteins, carbohydrates, lipids, nucleic acids]

lipids



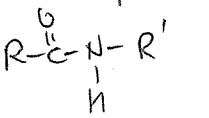
Ester

carbohydrates

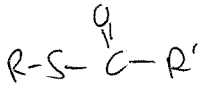


ether

proteins

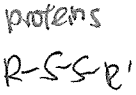


amide

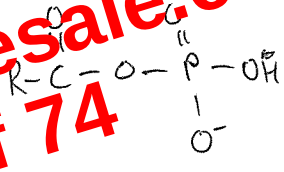


Thioester

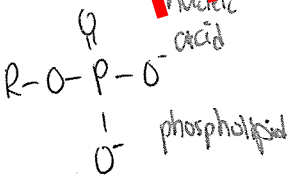
proteins



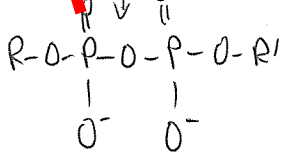
disulphide



mixed anhydride [carboxylic acid } phosphoric acid, (2 acids) ↳also acyl phosphate]

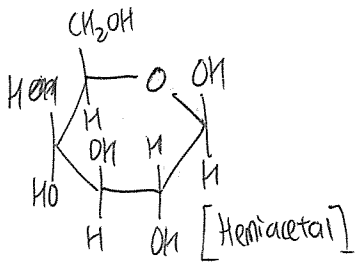


phosphate ester

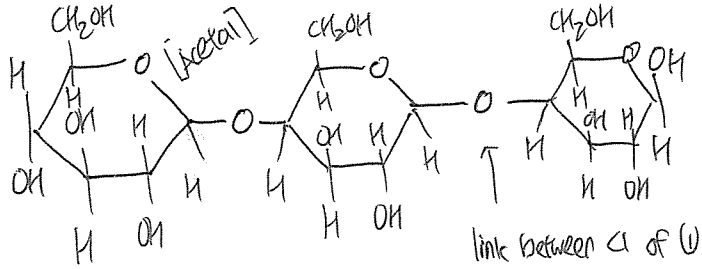


phosphoanhydride (H_2O removed)

Glycosidic bond between sugar ~~molecules~~ residues in oligosaccharides



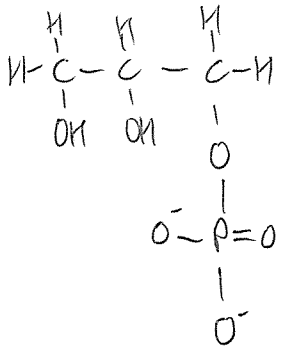
glucose



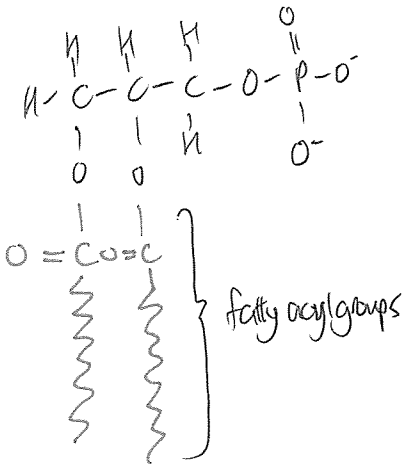
cellulose

link between C1 of (1) glucose } C4 of another

Ester bonds in fatty acids & phosphate ester bonds in metabolic intermediates

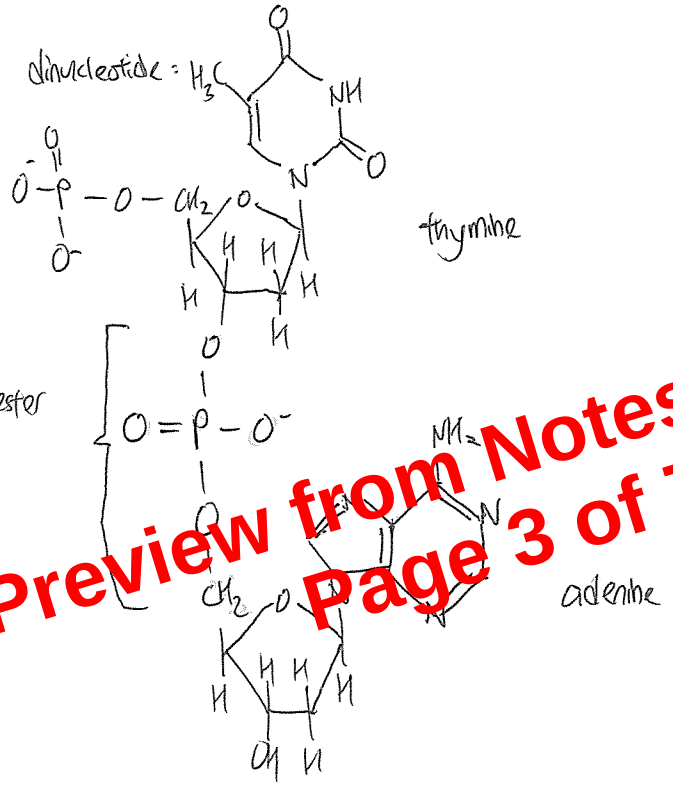


glycerol-3-phosphate



glycerophospholipid

Structure of a dinucleotide:

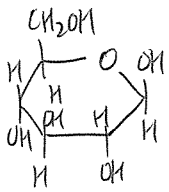


Solubility in water .... polar vs non polar

Polar molecules have a high proportion of polar / ionic groups

This makes them hydrophilic;

polar groups ↑ HBonding and solubility



glucose

- non polar molecules have few / no polar ionic groups
- hydrophobic because they minimise contact w water
- Entropic effect: water around hydrophobic molecule has reduced mobility

hexane

Water dissociation:

$H_2O + H_2O \rightleftharpoons H_3O^+ + OH^-$

hydronium ion, hydroxide ion

$H_2O \rightleftharpoons H^+ + OH^-$

equilibrium constant; $K_{eq} = \frac{[products]}{[reactants]} = \frac{[H^+][OH^-]}{[H_2O]}$

$= 1.8 \times 10^{-16} M = \frac{[H^+][OH^-]}{[55.5M]}$

water dissociation constant $K_w = 10^{-14} M^2$

$= [H^+][OH^-]$

$[H^+] = [OH^-] = 1 \times 10^{-7} M$

↝ lysosomes & vacuoles are slightly acidic pH ≈ 4

Søren Sørensen .... pH

pH of aqueous solution

acidic → $[H^+] = 10^{-3} M$ pH = 3

neutral → $[H^+] = 10^{-7} M$ pH = 7

basic/alkaline → $[H^+] = 10^{-10} M$ pH = 10 (×1000 [H⁺])

$pH = -\log_{10}[H^+]$

$K_w = 10^{-14} M^2 = [H^+][OH^-]$

$-\log 10^{-14} = pH + pOH$

$pH + pOH = 14$

↳ no matter what solute added, [water] of 55.5M is unchanged

Tertiary Structure of Globular Proteins:

- tertiary structure: the closely packed 3D form of a protein
- each protein folds its polypeptide chain into a 3° structure that is adapted for a particular biological function
- amino acids far apart in 1° structure may be brought together
- stabilised by non-covalent interactions eg: disulfide bridges
  - ↳ normally seen in extracellular proteins not in intracellular proteins

N-terminus → C-terminus
blue-green-yellow-red
cold → warm

Myoglobin - O_2 binding molecule in red blood cells
- largely α-helix

4 turns × 3.6 = 14 aa
0.15nm × 14 = 2.1nm

- hydrophobic residues in the interior of protein help stabilize the 3° structure of a protein

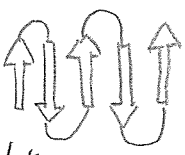
- Structure determination (X-ray, NMR) has revealed a huge variety of 3° structures

How do we make sense of the vast array of structures?

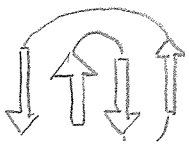
Inspection of structures can reveal folding patterns within them
- ↳ motifs or supersecondary structure
- ↳ Domains
- ↳ help in classification of protein folds

Supersecondary Structures (Motifs)
- ↝ recurring protein folding patterns
- ↝ comprising at least 2 connected 2° structure elements

a) Helix-loop-helix - 2 helices connected by a turn
b) coiled-coil - 2 amphipathic α-helices that interact in parallel through their hydrophobic edges
c) Helix bundle - several α-helices (3-5) that associate in an antiparallel manner to form a bundle
d) βαβ unit - 2 parallel β strands linked to an intervening α-helix by 2 loops
e) Hairpin - 2 adjacent antiparallel β strands connected by a β turn



f) β Meander - an antiparallel sheet composed of sequential β strands connected by loops / turns
g) Greek key - 4 antiparallel β strands (strands 1, 2 in the middle, 3 & 4 on the edge)
h) β sandwich - stacked β strands / sheets



Folding patterns help us classify protein structures

- Motifs & Domains help us begin to understand protein 3° structures through recognising 2° structure elements and their folding patterns

- The folding patterns help us classify protein structure into 4 categories, within which domain / motifs can be identified

Four categories of Protein Structure:

1) All α - Consist almost entirely of α helices & connecting loops

2) All β - Contain only β sheets & connecting loop structures

3) Mixed α+β - Contain motifs such as αβα units, where regions of α helix and β strand alternate or are interdispersed

4) α+β - Consist of local clusters of α-helices and β-sheet in separate, clearly distinct regions

Further Structural Classifiers

- Within each of the 4 main structural categories, a characteristic domain / fold may be identified and aid structure classification

- In addition to or instead of a domain fold, a folding motif may be added to the structure description

Quartenary Structure:

↝ Refers to the organization of subunits in a protein to multiple subunits

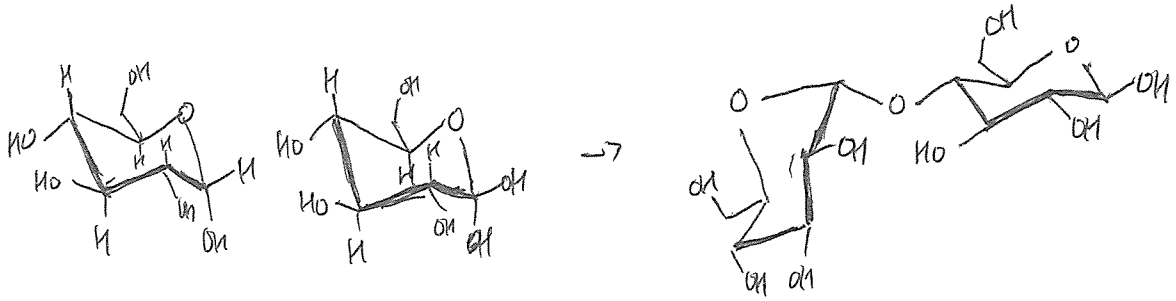
↝ Subunits have defined stoichiometry & arrangement

↝ Subunits associate through many weak non-covalent interactions

↝ Often a feature of regulated proteins eg: metabolic enzymes

↝ A multisubunit protein : oligomer

↝ If subunits are identical, dimers / tetramers predominate



* If β1,4 : straighter chain forming

α 1,4 = bent chain forming

Polysaccharides:

- Homoglycans : homopolysaccharides containing only (1) type of monosaccharide
- Heteroglycans : heteropolysaccharides containing ≥ more than (1) type of monosaccharide
- lengths and compositions of a polysaccharide may vary

* I⁻ trap in starch during iodine test

Polysaccharides: glucose storage

→ D-glucose is stored intracellularly in polymeric forms

→ Plant } fungi : starch

→ animals : glycogen

→ Starch is a mixture of amylose } amylopectin

Amylose linked by α1,4 glycosidic bonds ; Amylopectin has α1,6 linkages

Amylose – compact helix / spiral

Amylopectin : chain with spiral as in amylose but have branches due to α1,6-glycosidic bonds

↳ branches every 24-30 glucose units

↳ branching ↑ compactness

↳ ↑ no. of chain ends – faster formation } degradation

Glycogen

↝ very similar to amylopectin but usually larger (↑ glucose units)

↝ ↑ branches (every 8-12 residues)

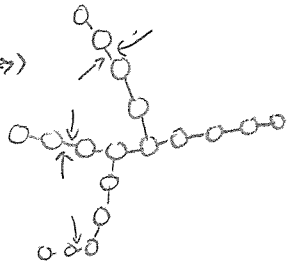
↝ more compact structure } faster metabolism

↑ branches, faster access to sugar

α-amylase (endoglycosidase) - catalyze random hydrolysis of α1,4 glycosidic bonds in amylose / amylopectin

β-amylase (exoglycosidase) - acts on terminal glycosidic bonds

- catalyzes sequential release of maltose (→)



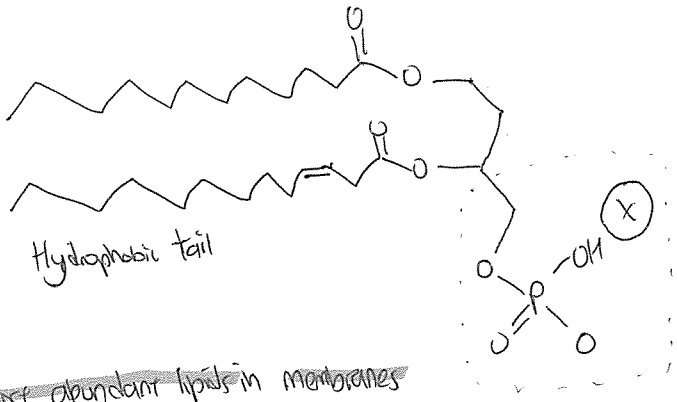
Cis double bonds introduce kinks:

- less intermolecular van der waals interaction
- more fluid
- healthier than 'trans' form as our body can utilise cis molecules for lipid synthesis

Triacylglycerols:

- Fatty acids are an important fuel source — stored as neutral lipids → triacylglycerols (TAGs)
- TAGs composed of 3 fatty acyl residues esterified to a glycerol
- very hydrophobic, stored in cells in an anhydrous form eg: in fat droplets

Membrane lipids: glycerophospholipids → amphipathic



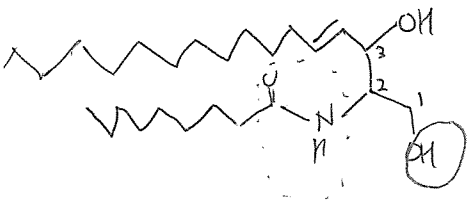
X=H	phosphatidate
X= ethanolamine	phosphatidyl ethanolamine
choline	phosphatidyl choline
serine	phosphatidyl serine
inositol	phosphatidylinositol

- most abundant lipids in membranes
- possess a glycerol backbone
- 2 fatty acids esterified to glycerol
- 1 phosphate esterified to 3rd carbon in glycerol
- The phosphate group can be further esterified to a polar group (X)

Glycerophospholipids hydrolysed by phospholipase A_1, phospholipase A_2

phospholipase D converts glycerophospholipids to phosphatidates

Membrane lipids: sphingolipids



→ a large family of membrane lipids

→ instead of glycerol backbone, like TAG & phospholipids, they have a sphingosine unit [C18 alcohol with a trans C=C at C4 & C5]

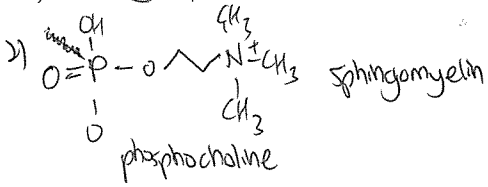
→ like phospholipids, have 2 non-polar tails & a polar head group (amphipathic)

→ 1 fatty acid is amide linked

→ abundant in CNS

SPHINGOLIPIDS

1) O=H ceramide

2)  sphingomyelin

3) glucose/galactose — cerebroside

4) Complex oligosaccharide — ganglioside

* glucose/galactose β1,4 glycosidic linked to O of ceramide

Sphingomyelin → phospholipid as it contains phosphate

cerebroside & ganglioside → ~~glycosphingolipids~~ glycosphingolipids

Methods for protein purification:

1. Disruption of cells / tissues
2. Differential centrifugation to isolate cellular organelles
3. Separation of proteins based on
   - Solubility, size, charge, hydrophobicity, affinity for ligands

Techniques used for the physical disruption of cells:

* the smaller the cell, the harder it is to break *

Lysis Method	Apparatus	Description
Mechanical	Waring Blender, Polytron	Rotating blades grind and disperse cells / tissues
Liquid Homogenization	Dounce Homogenizer, French Press	cell / tissues suspensions are sheared by forcing them through a narrow space
Sonication	Sonicator	High frequency sound waves shear cells
Freeze / thaw	freezer or dry ice, ethanol	repeated cycles of freezing & thawing disrupt cells through ice crystal formation [ice crystals penetrate the cell membrane]
Manual Grinding	Mortar and Pestle	Grinding plant tissue, frozen in liquid nitrogen

* water-freeze as pure molecules, expanding & disruption of cells

Pressure — shear forces

- liquid passing through the centre is faster than at the side due to frictional forces
- High pressure needed to force fluid through the narrow tube
- gases dissolved in liquid will move out leading to cavitation
  - The French Press

Separation of organelles from animal cells by differential centrifugation:

xg, g = 9.81 m/s²

F = −ω²r

ω = angular velocity (radians/s)

= 2πrpm / 60, where rpm = revs per minute

r = radius in metre

F / 9.81 = xg

100g ↓x speed, ↓x radius 200g

400g

Sonication dissolving gas ↑ pressure

low pressure — gas leaving (cavitation)

Centrifugal force ↑ according to the square of the angular velocity

Eg: 500g, r = 10cm, ω = ?, rpm = ?

F = ω²r

500 × 9.81 = ω² (0.1)

ω = 700 radian/s × 60

= 42,000 radians/m → / 2π = 6684 rpm

(10min) 500g – separate nuclear fraction + unbroken cells

10,000g (20minutes) – separate mitochondrion (1-2μm)

100,000g (1hour) – separate microsomal fraction [broken cell membranes including organelle, RM ... etc]

Mass spectometry can provide accurate mass determination

- Identification of potens by mass spectrometry
  - ↓mass, ↑charge move fastest
  - TOF depends on mass/charge

→ fragment the isolated protein using a protease eg: trypsin cleaves after Lys / Arg

→ compare fragment pattern w theoretical trypsin digest

→ MALDI : matrix assisted laser desorption / ionization } suitable for
TOF = time of flight } proteins

Exploring the Proteome

- 2D gel electrophoresis used to separate cellular potens
- mass spectrometry used to identify protein spots from their fragment mass spectrum after proteolysis
- Comparisons in levels of potem expression can be made between cells / tissues treated w drugs or between normal } diseased tissue
- Proteome analysis can be used to study developmental changes

Proteome → entire set of potens expressed by a genome / cell / tissue / organism at a certain time under defined conditions.

Antibodies – Powerful tools to detect potens

1) Polyclonal Antibodies – mixed population of antibody molecules that recognise diff. epitopes on antigen

2) Monoclonal Antibodies – uniform population of antibodies that recognise the same epitope on the antigen

Western Blot analysis:

SDS-PAGE gel → Transfer potens onto polymer sheet → Add labelled specific antibody. Wash to remove unbound antibody. Antibody on polymer sheet will react w antibody → overlay photographic film. Expose & develop → Protein band detected by specific antibody on a autoradiogram

* These days, antibodies are usually labelled w an enzyme / luminescent molecule rather than using radiolabelled antibody

Indirect Immunofluorescence → Antibody is labelled w a fluorescent molecule and then visualised w fluorescent microscope

Immunogold labelling of thin sections → antibody is labelled w a gold colloidal particle and then visualized with an EM.

The Citric Acid Cycle

- aerobic catabolism [oxidation reactions]
- intermediates used for biosynthesis reactions
- takes place in the mitochondria in eukaryotes / cytosol in bacteria

Energy in the citric acid cycle:

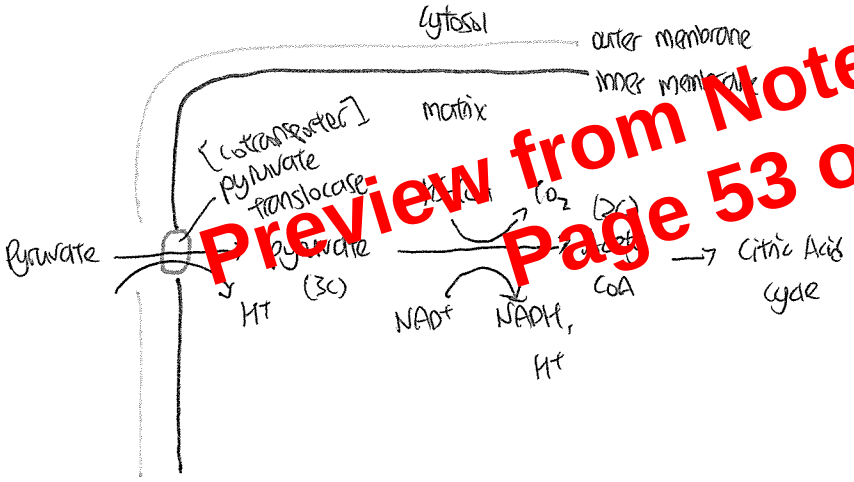
- Energy of the oxidation reactions is largely conserved as reducing power
- Coenzymes reduced

  $NAD^+ \rightarrow NADH$

  Ubiquinone (Q) $\rightarrow$ Ubiquinol (QH_2)

Entry of Pyruvate into mitochondrion

- Pyruvate ~~translocate~~ translocase transports pyruvate into the mitochondria in symport w H^+
- Pyruvate is then coupled to coenzyme A and decarboxylated and oxidised by a huge enzyme complex named pyruvate dehydrogenase



Citric Acid Cycle

For each acetyl CoA which enters the cycle

1) 2 molecules of CO_2 are released
2) Coenzymes NAD^+ and Q are reduced
3) One GDP / ADP is phosphorylated
4) The initial acceptor molecule (oxaloacetate) is reformed

Overall equation for gluconeogenesis:

2 pyruvate + 2NADH + 4ATP + 2GTP + $4H_2O$ + $2H^+$ → Glucose + 2NAD$^+$ + 4ADP + 2GDP + 6P$_i$

Note that it costs much more energy to generate glucose from pyruvate than can be generated from glycolysis.

This is because neither process is anywhere near 100% efficient

↳ glycolysis only generate 2ATP

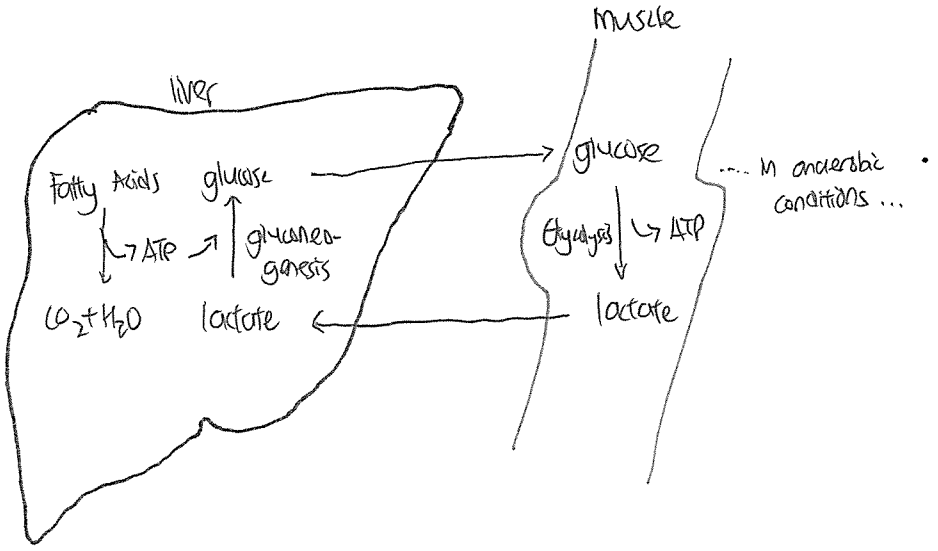
Pyruvate carboxylase:

- This important enzyme is at start of gluconeogenesis
- catalyzes a metabolically irreversible reaction
- allosterically activated by acetyl CoA
- Accumulation of acetyl CoA from fatty acid oxidation signals abundant energy and directs pyruvate to oxaloacetate for gluconeogenesis
  ↳ too much acetyl CoA as it is no longer needed for ATP production, hence it is directed to glucose synthesis

Precursors for Gluconeogenesis

- Any metabolite that can be converted to pyruvate / oxaloacetate
- major gluconeogenic precursors in mammals
  1) lactate
  2) most amino acids (especially analine)
  3) glycerol (from [illegible] hydrolysis)

The Cori cycle: the interaction of glycolysis and gluconeogenesis



Reactions of β-oxidation:

- one round of β-oxidation: 4 enzyme steps produce acetyl CoA from fatty acyl CoA
- Each round generates one molecule of each
  - QH_2 (ubiquinol)
  - NADH
  - Acetyl CoA → can enter citric acid cycle
  - Fatty Acyl CoA (2 carbons shorter each round)

Net yield of ATP per palmitate oxidized to 16 CO_2 (palmitate → 16 carbon long, saturated without any double bonds)

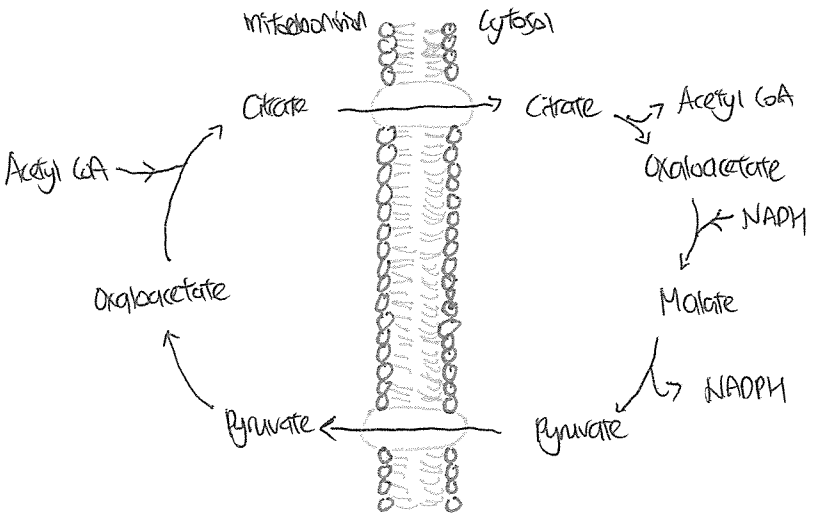
	ATP generated
8 acetyl CoA	80
7 QH_2	10.5
7 NADH	17.5
	108 ATP
ATP needed to activate palmitate	-2ATP
Net yield →	106 ATP

16 carbon fatty acid → 7 rounds of β-oxidation
[16-14-12-10-8-6-4-2]

* 2ATP needed to activate any fatty acid, regardless of length
Hence, the longer the fatty acid, the greater the efficiency, the greater the amount of ATP generated

Fatty Acid Synthesis:

- occurs mainly in liver and adipocytes (mammals)
- when glucose is plentiful, excess acetyl CoA is produced by glycolysis — used for fatty acid synthesis
- glucose oxidation in the pentose phosphate pathway provides NADPH for fatty acid synthesis
- synthesis occurs in the cytosol



* NADPH usually found in cytosol