GLYCOLYSIS

1. Aerobic

1 Glucose (6c) $\xrightarrow[\text{steps}]{10}$ 2 Pyruvate (3c)

2. Anaerobic

1 Glucose $\xrightarrow[\text{steps}]{11}$ 2 Lactate

$C_6H_{12}O_6$ $\xrightarrow{10\text{ steps}}$ 2 $CH_3-C(=O)-COOH$ $\xrightarrow{11\text{ steps}}$ 2 $CH_3-CH(OH)-COOH$

Free energy → ATP

Pyruvate

Lactate

GLUCOSE

↓ Glycolysis

2 Pyruvate

Anaerobic → 2 Ethanol + $2CO_2$ — e.g. Alcohol fermentation by Yeast

O_2 / CO_2 Aerobic → 2 AcetylCoA

O_2 → TCA cycle → $4CO_2 + 4H_2O$

e.g. Animal, Plant and Microbial cells under aerobic conditions.

Anaerobic → 2 Lactate — e.g. Active Muscle

Anaerobic glycolysis in an active skeletal muscle

Sum:

(iii) $Glucose + 2Pi + 2ADP \longrightarrow 2Lactate^- + 2H^+ + 2ATP + 2H_2O$

(i) $Glucose \longrightarrow 2Lactate^- + 2H^+$

$\Delta G^{o'} = -47$ Kcal/mol

(ii) $2Pi + 2ADP \longrightarrow 2ATP + 2H_2O$

$\Delta G^{o'} = +14.6$ Kcal/mol

$\Delta G_s^{o'} = -47 + (+14.6) = -32$ Kcal/Mol

Overall STD-free energy change of glycolysis.

But;

$Glucose \longrightarrow 2Lactate^- + 2H^+ \quad -47$ kcal/mol

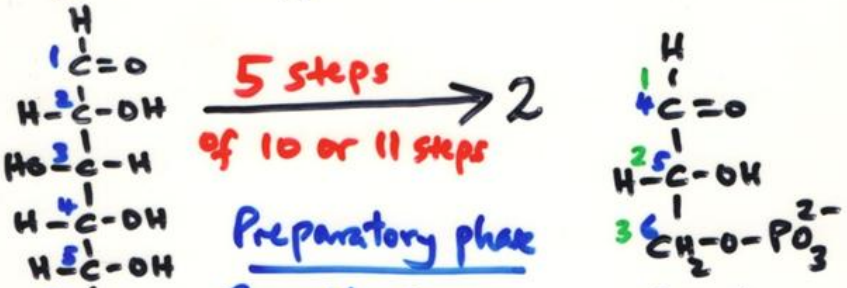
$Glucose + 6O_2 \longrightarrow 6CO_2 + 6H_2O \quad -686$ kcal/mol

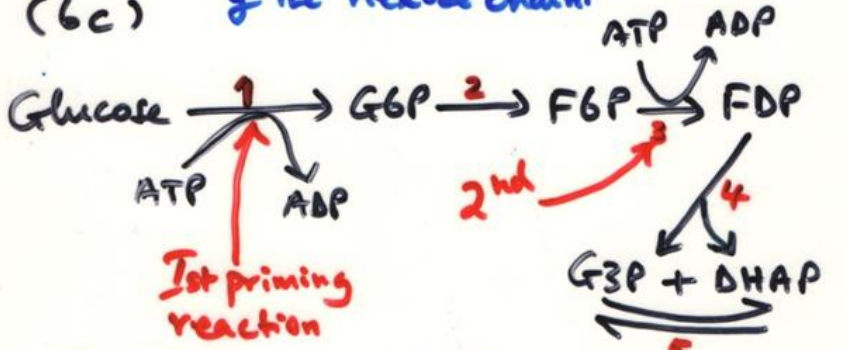
$\therefore \frac{47}{686} \times 100 \simeq 7\%$ Glycolysis produces only about 7% of the energy inherent in a glucose residue.

2 Lactate contain most of the energy which can only be released by complete oxidation to $CO_2 + H_2O$ with O_2 as the oxidant.

GLYCOLYSIS has 2 Phases;

1st Phase ← Investment phase – Involves phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate (G3P).



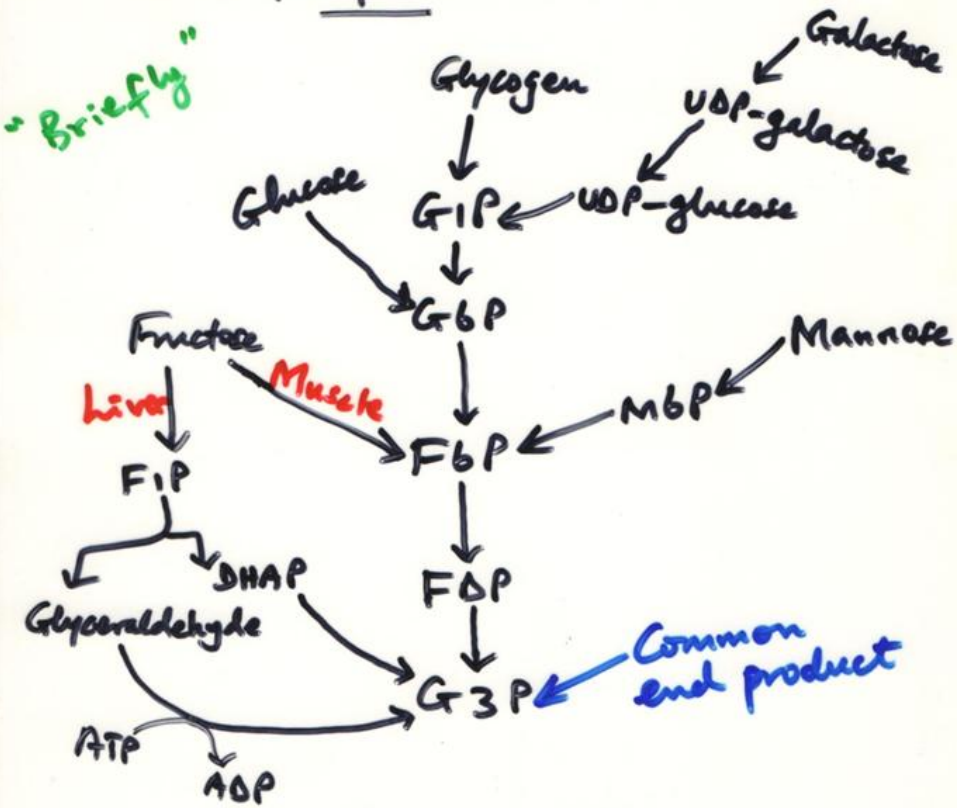


2ATPs utilized to fix a phosphate group at C6 and C1' of glucose respectively.

Other hexoses i.e. Fructose, Galactose and Mannose also enter the preparatory phase and end up being converted to G3P. G3P is the common product of all hexoses – Discussed later (in detail)

Entry of glycogen and different hexoses in the preparatory phase (Ist stage) of glycolysis.

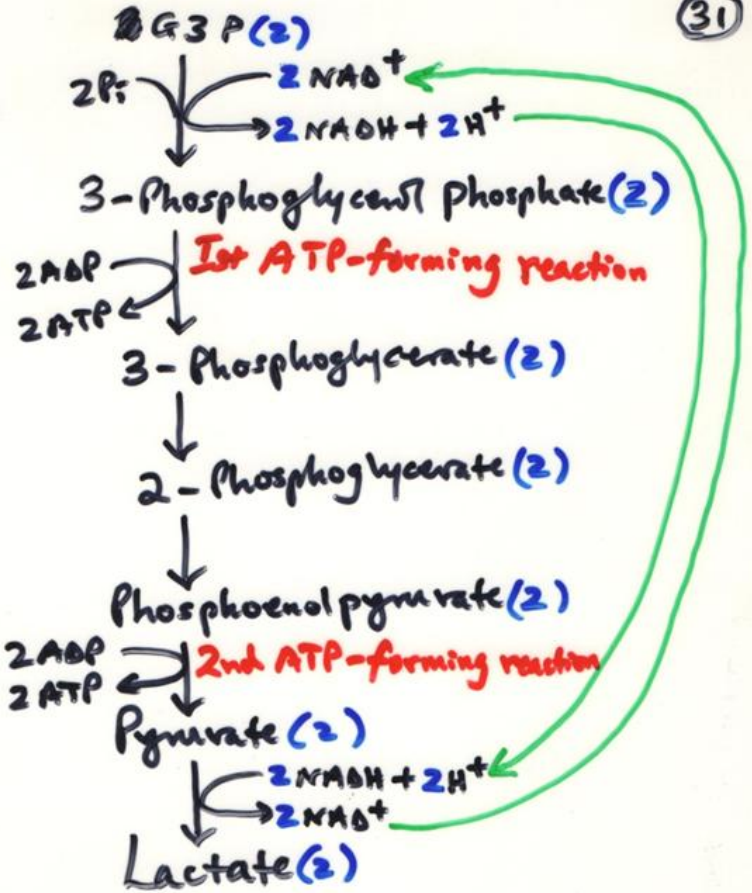
"Briefly"



2nd Phase – "Gain phase"

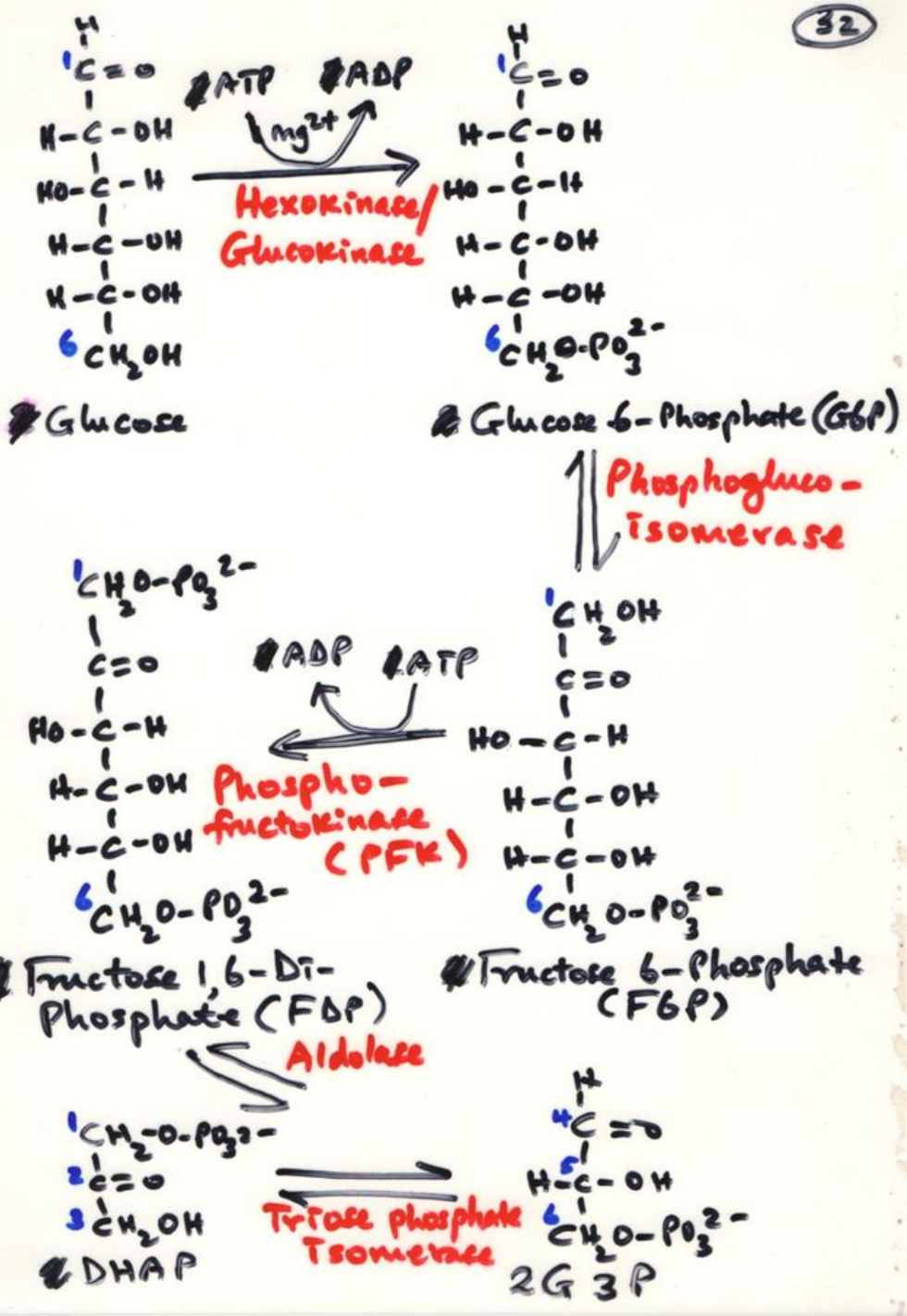
5 or 6 steps where the energy (ATP) used to form G3P in the Ist phase is repaid. The two molecules of G3P is converted to two molecules of Pyruvate or Lactate depending on the availability of O_2.

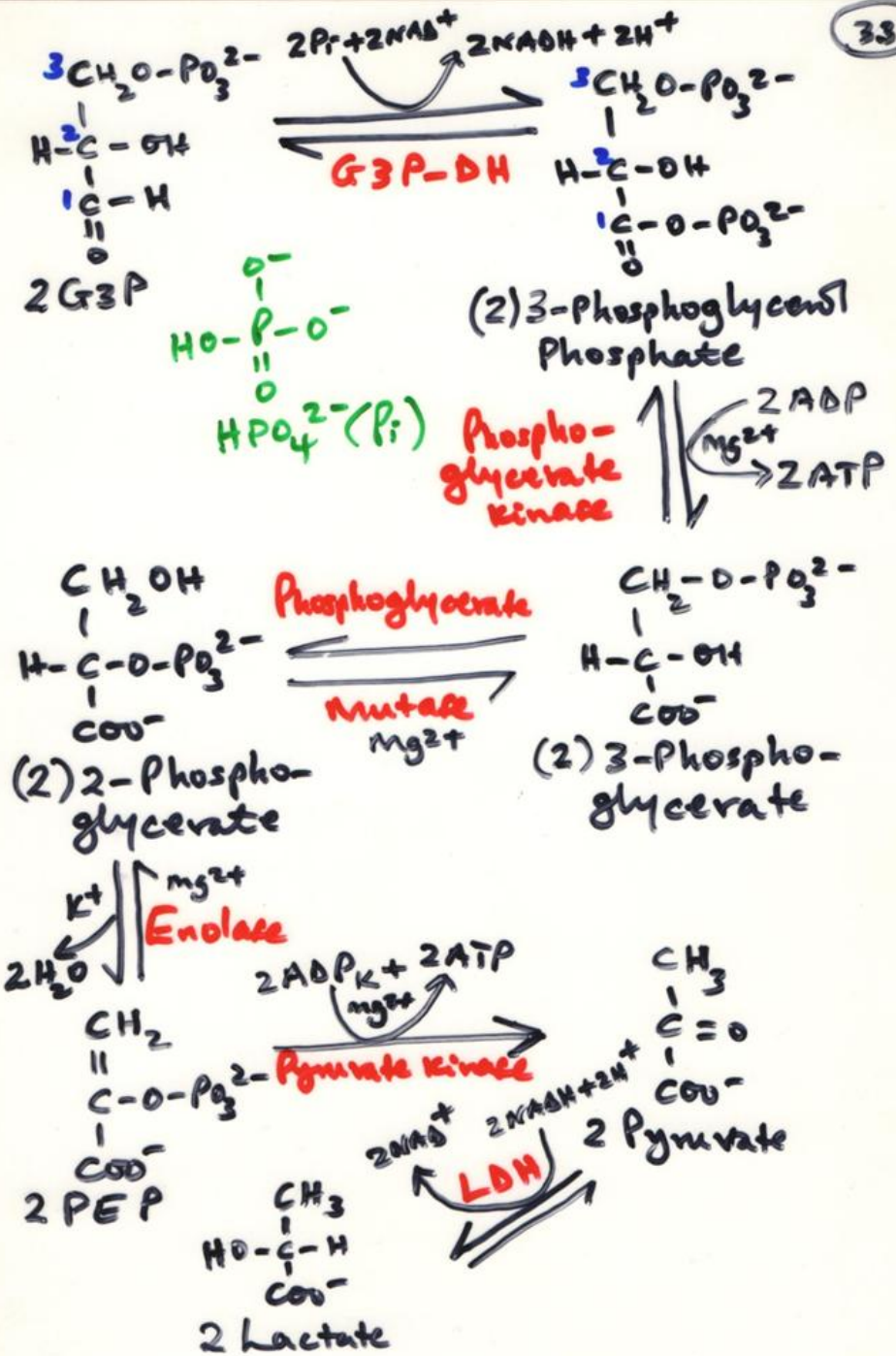
Gain = 2 ATPs (overall) from 4 ATPs formed.



3 different chemical transformations take place during glycolysis;

1) Pathway of carbon atoms – degradation
2) Pathway of phosphate groups – Phosphorylation.
3) Pathway of atoms / electrons – Transfer

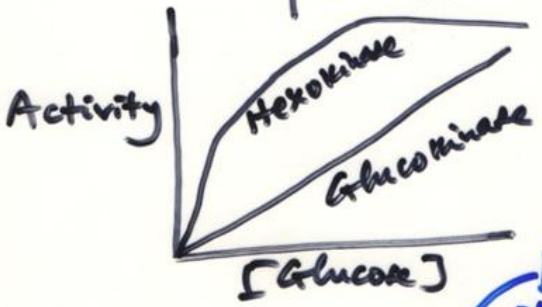

ATP
ADP
Mg^{2+}
Hexokinase/
Glucokinase
Glucose
Glucose 6-Phosphate (G6P)
Phosphogluco-
isomerase
ADP
ATP
Phospho-
fructokinase
(PFK)
Fructose 1,6-Di-
Phosphate (FDP)
Fructose 6-Phosphate
(F6P)
Aldolase
DHAP
Triose phosphate
Isomerase
2G3P


2Pi + 2NAD+
2NADH + 2H+
G3P-DH
2 G3P
(2) 3-Phosphoglycerol Phosphate
HPO4 2- (Pi)
Phospho-glycerate kinase
2ADP
Mg2+
2ATP
Phosphoglycerate
Mutase
Mg2+
(2) 2-Phospho-glycerate
(2) 3-Phospho-glycerate
K+
2H2O
Mg2+
Enolase
2 PEP
2ADP + 2ATP
Mg2+
Pyruvate kinase
2NAD+
2NADH + 2H+
LDH
2 Pyruvate
2 Lactate

DETAIL - Some reactions

1. Glucose + ATP $\xrightarrow{\text{Hexokinase / Glucokinase}}$ G6P + ADP

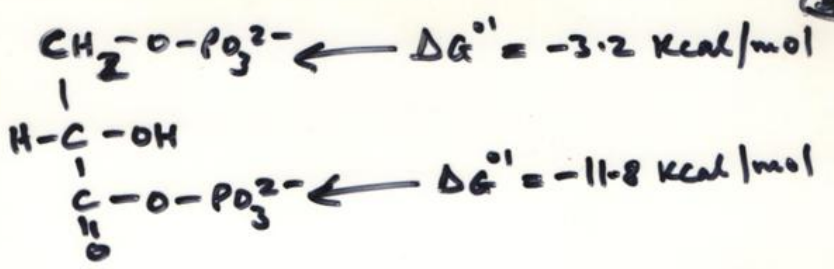
	Hexokinase	Glucokinase
- Located in:	Muscle	Liver
- K_m for glucose	0.1 mM	10 mM
- Acts when:	[Glucose] < 5 mM	> 5 mM
- Phosphorylation	All hexoses except Gal.	Only Glucose
- Inhibited by:	G6P	Not
-	Not deficient	Deficient in Diabetes Mellitus
	Isoenzymes	

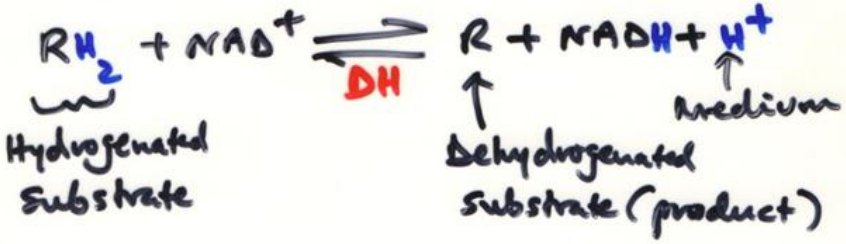


6. G3P + NAD^+ + P_i $\rightleftharpoons$ 3-Phosphoglycerol phosphate + NADH + H^+

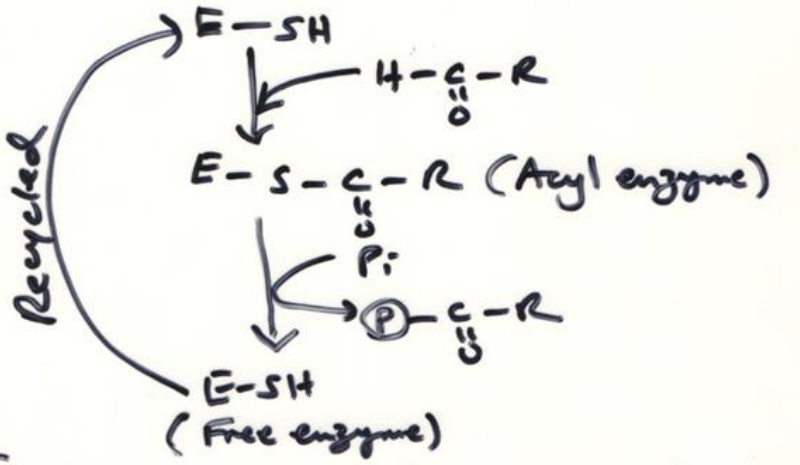
Dehydrogenation of G3P

Carboxylic anhydride (acyl phosphate)





G3P-DH; Has an essential sulfhydryl (-SH) group which forms a thioester linkage with the substrate (G3P)

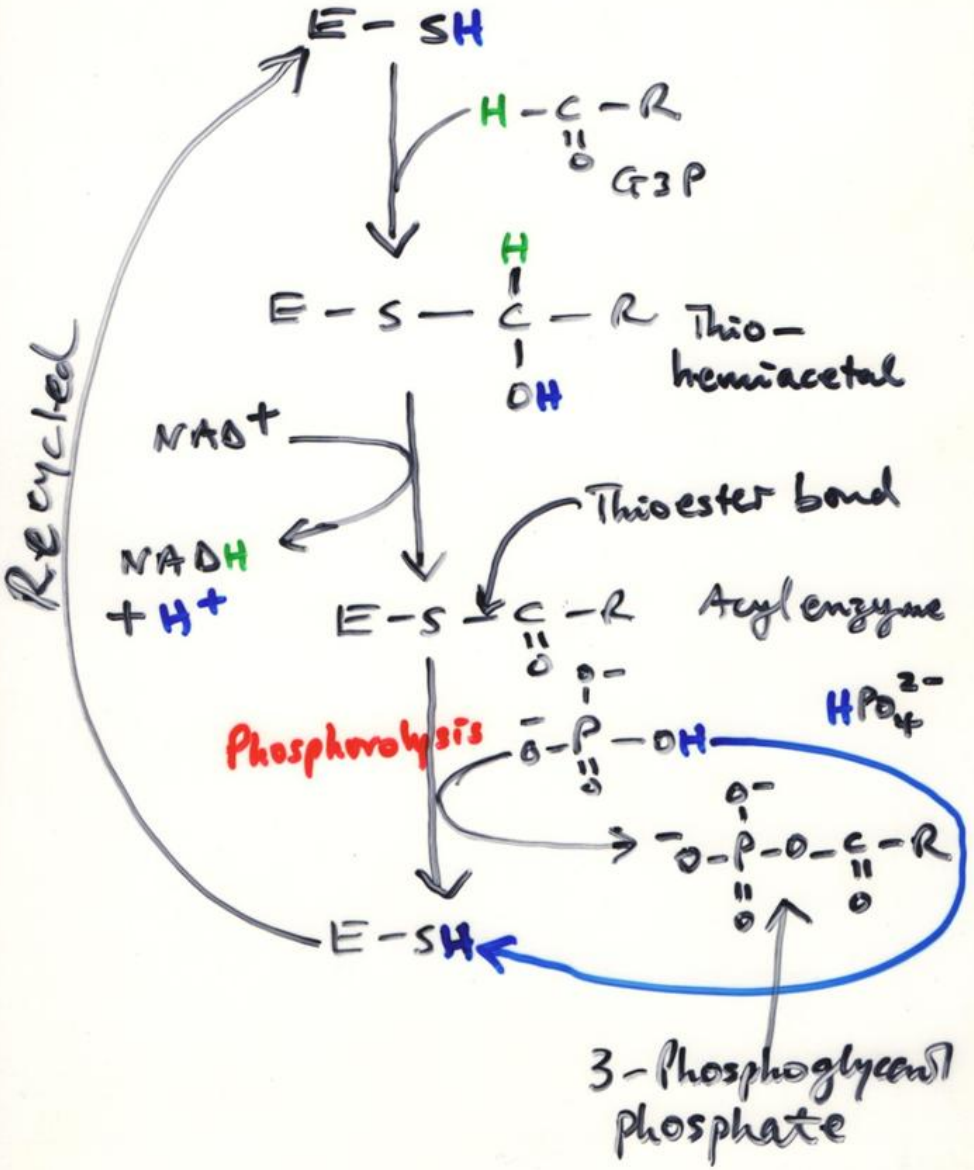


It is inhibited by Iodoacetamide or Iodoacetate.

$E-SH + ICH_2COO^- \rightarrow E-S-CH_2COO^- + HI$

Active — Inactive

Reaction mechanism of G3P-DH.



7. 3-Phosphoglyceraldehyde $\xrightleftharpoons[ATP]{ADP}$ 3-Phosphoglycerate

The above reaction is an example of Substrate-Level phosphorylation.

- i.e. generation of ATP from a substrate with high energy and the reaction catalysed by a soluble enzyme.

10. $R\text{-}OPO_3^{2-} \xrightarrow[ATP]{ADP} R\text{-}OH$

$PEP + ADP \longrightarrow Pyruvate + ATP$

Another example.

Overall balance sheet for anaerobic glycolysis;

$Glucose + 2ATP + 2P_i + 2NAD^+ + 2NADH + 2H^+ + 4ADP \longrightarrow$

$2Lactate^- + 2H^+ + 4ATP + 2H_2O + 2NADH + 2H^+ + 2NAD^+ + 2ADP$

Cancel out;

$\therefore Glucose + 2P_i + 2ADP \longrightarrow 2Lactate^- + 2H^+ + 2ATP + 2H_2O$

(Overall equation for anaerobic glycolysis. $\Delta G^{o\prime} = -32$ Kcal/mol

Overall balance sheet for aerobic glycolysis;

$$\text{Glucose} + 2ATP + 2P_i + 2NAD^+ + 4ADP \longrightarrow 2\,\text{Pyruvate}^- + 4ATP + 2H_2O + 2NADH + 2H^+ + 2ADP + 2H^+$$

$$\therefore \quad \Delta G^{o\prime} = -20.4 \text{ kcal/mol}$$

$$\text{Glucose} + 2P_i + 2NAD^+ + 2ADP \longrightarrow 2\,\text{Pyruvate}^- + 2ATP + 2H_2O + 2NADH + 2H^+ + 2H^+$$

NB The two NADH formed by dehydrogenation of (2)G3P are not re-oxidized by pyruvate in the LDH reaction.

The two NADH are channeled to the Electron-Transport Chain (ETC) in the Matrix where they reduce O_2 to H_2O.

To be discussed later

$$2NADH + 2H^+ + O_2 \longrightarrow 2NAD^+ + 2H_2O$$

$3ADP + 3P_i \quad 3ATP$

Q. What is the fate of the other hexoses?

1. Fructose

(a) Muscle / Kidney

Fructose + ATP $\xrightarrow[\text{Hexokinase}]{Mg^{2+}}$ F6P + ADP + H^+

(b) Liver

Fructose + ATP $\xrightarrow{\text{Fructokinase}}$ F1P + ADP + H^+

F1P $\rightleftharpoons$ (Aldolase) Glyceraldehyde + DHAP

Glyceraldehyde + ATP $\xrightarrow{\text{Triose Kinase}}$ G3P + ADP + H^+

DHAP $\rightleftharpoons$ (T.P. isomerase) G3P

G3P + G3P → 2 molecules coming from 1 molecule of Fructose

2 molecules → Glycolysis

2. Mannose

Mannose + ATP $\xrightarrow[\text{Hexokinase}]{Mg^{2+}}$ M6P + ADP + H^+

M6P $\rightleftharpoons$ (Phosphomanno-isomerase) F6P

F6P → Glycolysis

3. Galactose

Galactose + ATP $\xrightarrow[\text{Galactokinase (GALK)}]{Mg^{2+}}$ Galactose 1-Phosphate + ADP + H^+

- UDP-glucose = functions as a coenzyme-like carrier of glucosyl residues.
- UDP = carrier of hexose groups.

Galactose 1-Phosphate

UDP-Gl. → G1P: UDP-Gl/galactose 1-phosphate uridylyltransferase = GALT

UDP-galactose

UDP-galactose 4'-epimerase = GALE

UDP-glucose

PP: → UTP: UDP-glucose pyrophosphorylase

G1P

Glycolysis

Phosphoglucomutase

G6P

Leloir Pathway

Q. Discuss the metabolic disorders associated with galactose in humans.

1. Galactosemia – 3 types/forms

Enzyme: UDP-glucose/galactose 1-phosphate uridylyl transferase is genetically defective.

1/60,000 infants

∴ ↑[Galactose] → Galactitol in blood and tissues.

↑[Galactose 1-phosphate]

Manifestations; (In infants due to milk ingested.)
- Liver enlargement
- Cataracts – vision impaired
- Mental retardation

Treatment: Withholding milk from the diet – or other milk products.

Other forms of galactosemia;
(i) Defective galactokinase
(ii) Defective UDP-galactose 4-epimerase.

2. Lactose intolerance – 3 types

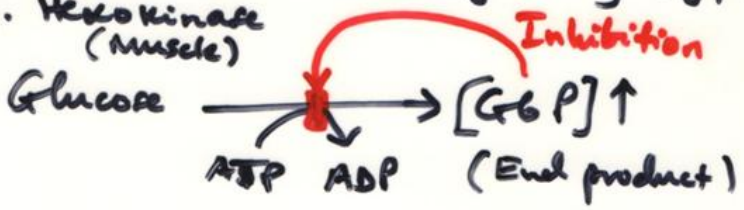
Found in adults of Asian & African origin = mainly.
- Defective (lack of) Lactase in small intestine after childhood.

→ Diarrhoea + intestinal gases – H_2, CO_2, methane.

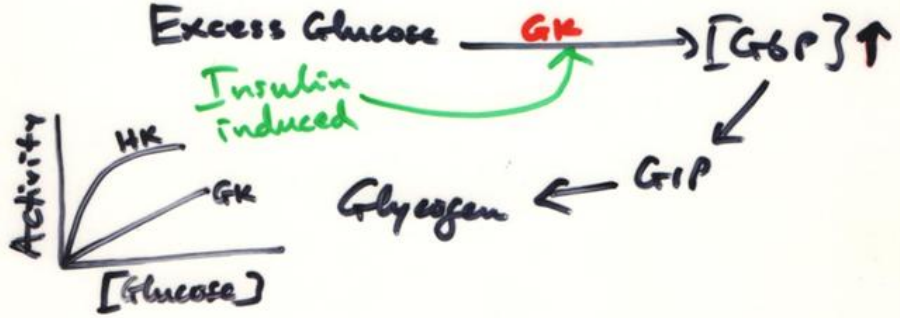
Q. How is the entry of glucose residues into the glycolytic pathway regulated?

1) By phosphorylation at C6 by ATP in the Hexokinase or glucokinase reaction. The two enzymes are regulatory enzymes.

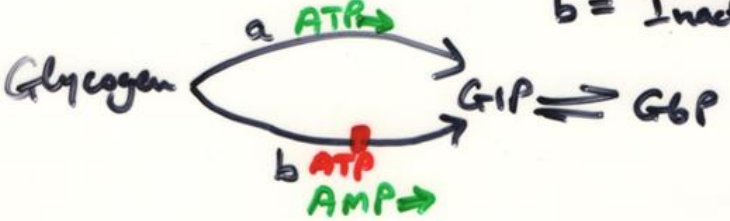
e.g. Hexokinase (Muscle)



e.g. Glucokinase (liver)



2) By the action of glycogen phosphorylase
It exists in 2 forms < a = active
b = "Inactive"



REGULATORY ENZYMES

Enzyme systems have a pacemaker or regulatory enzyme;

1) Catalyzes the slowest or rate-limiting step.
2) Has catalytic activity like any other enzyme.
3) It is capable of increasing or decreasing the catalytic activity of other enzymes – it is capable of receiving signals from the environment.

Two classes

1) Noncovalently regulated enzymes (Allosteric)
2) Covalently " "

NON-COVALENT

Regulated by noncovalent binding of modulator molecules (Effector) < + −

e.g. End-product inhibition (Feedback)

Glucose —HK→ G6P

↖ Negative modulator

* Reversible

COVALENT

* Reversible covalent modification of the enzyme molecule

e.g. Glycogen phosphorylase (GP) in Muscle/ Liver

$$(\text{Glucose})_n + Pi \xrightarrow{GP} (\text{Glucose})_{n-1} + G1P$$

Glycogen chain — Shortened glycogen chain

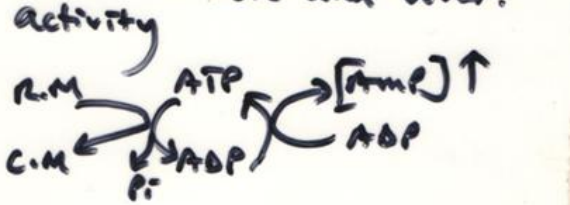
GP

- Has 2 subunits with an essential serine residue in its active site.
- The 2 forms differ in their quaternary structure.
- It is also regulated noncovalently by AMP (+ modulator of "b" form).
- Regulated covalently by ATP (+ modulator of "a" form).

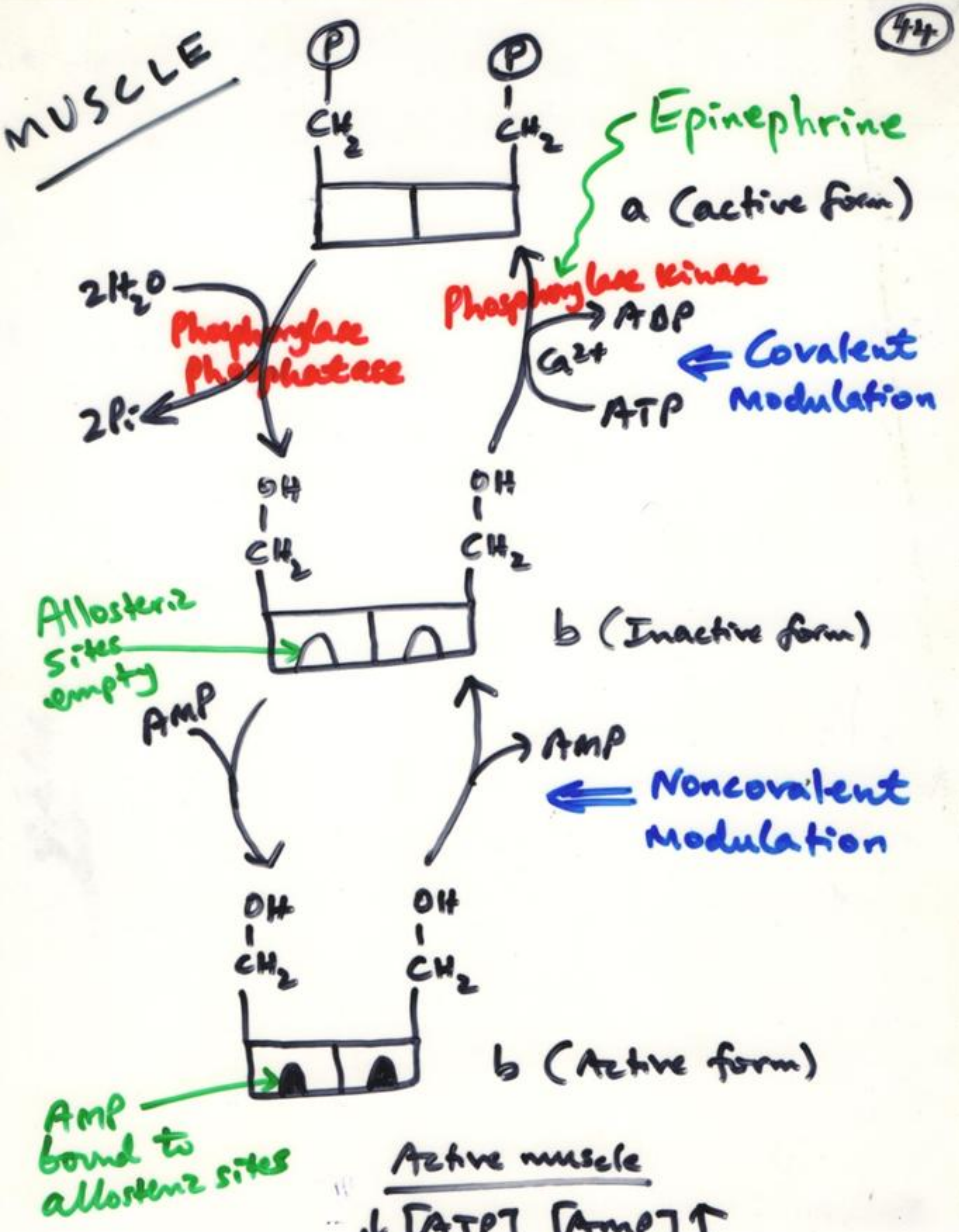
Q. Discuss the regulation of glycogen phosphorylase in muscle and liver.

a) Muscle

(i) Active



MUSCLE

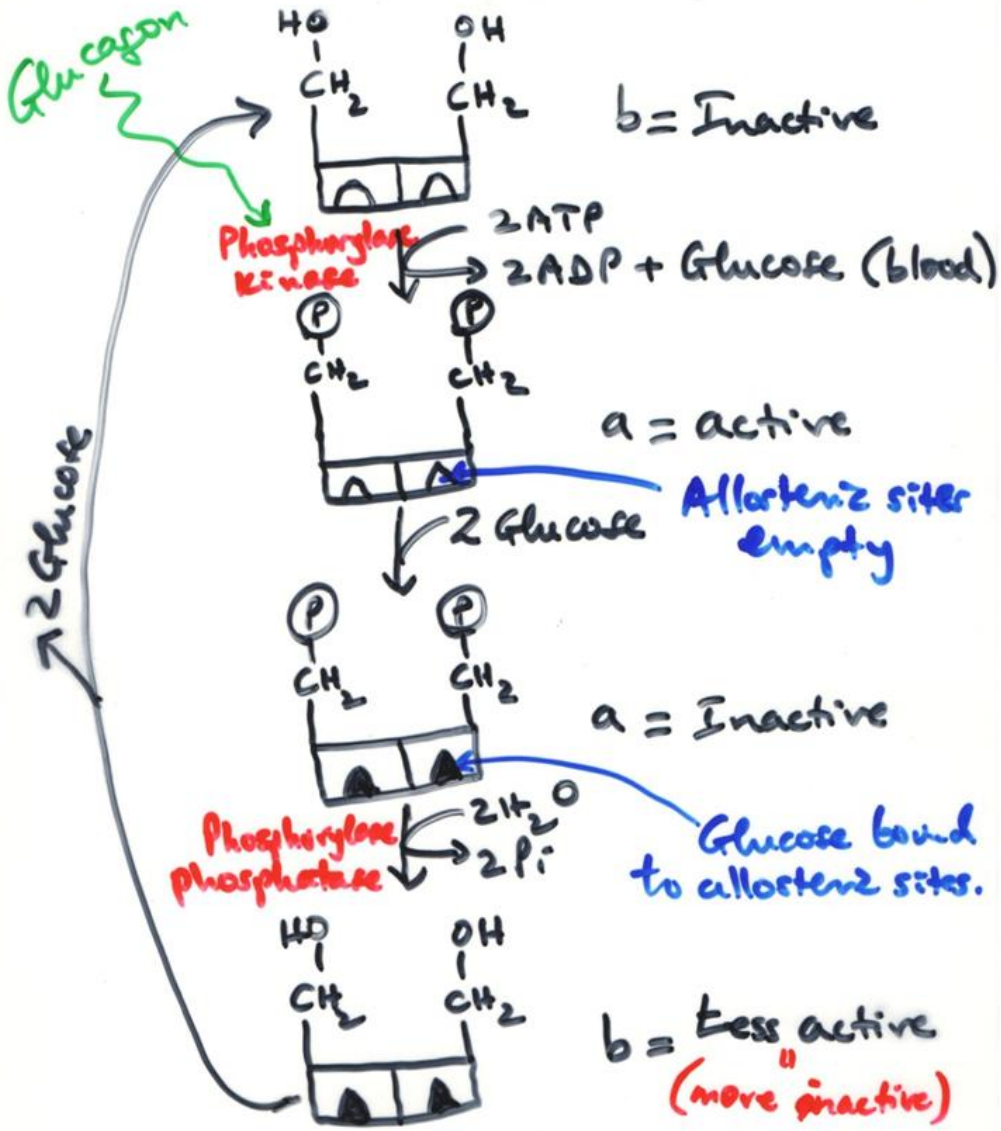


Active muscle

↓ [ATP] [AMP] ↑

Present in a form then switches to b (active form)

LIVER GP is regulated allostenically and hormonally;



Resting muscle

GP is in b (inactive) form because [ATP] > [AMP]. ATP acts a – modulator of b form – making GP exist in its inactive form.

∴ ATP regulates the activity of GP both by covalent and by noncovalent modulation.

Q. How is the glycolytic sequence regulated ?

1) PFK

Activators	Inhibitors
AMP	ATP
FDP	Citrate
ADP	Mg^{2+}
P_i^-	Ca^{2+}
K^+	

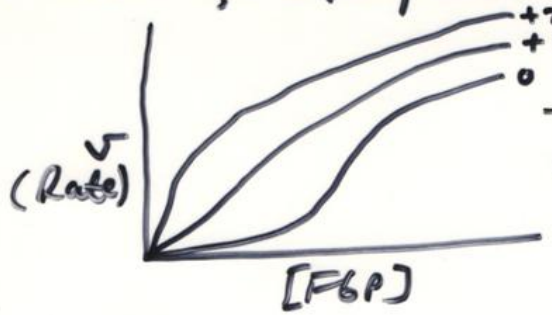
Fructose 2,6 Bisph.

Activators

When [P_i], [ADP] [AMP] is High, [ATP] low – favors synthesis of FDP.

F6P + ATP ⟶ FDP + ADP

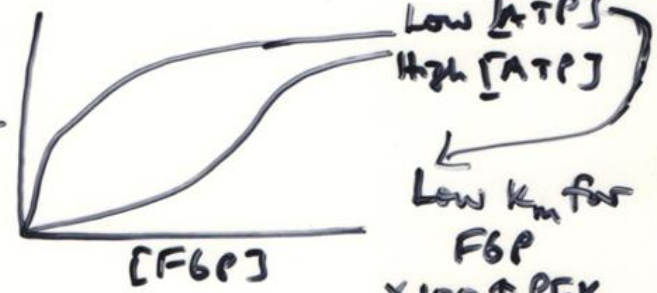
Fructose 2,6 Diphosphate — Increases PFK affinity for F6P
— Diminishes the inhibitory effect of ATP.



Inhibitors

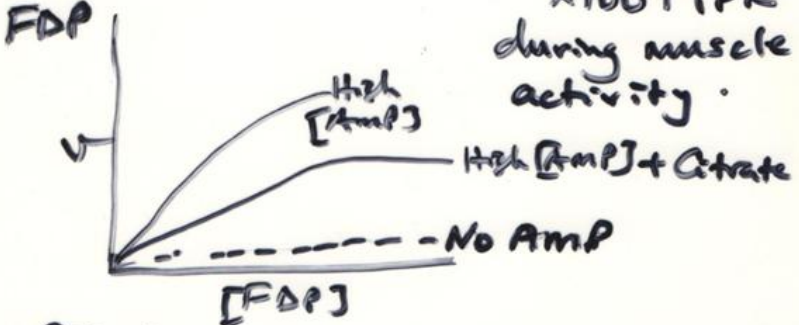
ATP

Allosteric regulation



Low K_m for F6P

×100↑ PFK during muscle activity.



Citrate = TCA cycle intermediate

∴ [ATP] and [Citrate] is high, PFK is slowed (inhibited). The cell has enough fuel and energy which is not in use eg. Resting muscle.

THE CITRIC ACID CYCLE / THE TRICARBOXYLIC ACID CYCLE / THE KREBS CYCLE

Glycolysis — how cells obtain energy (ATP) from CHOs in the "absence" of O_2. The pathway is unable to tap all the energy trapped in CHOs e.g. glucose.

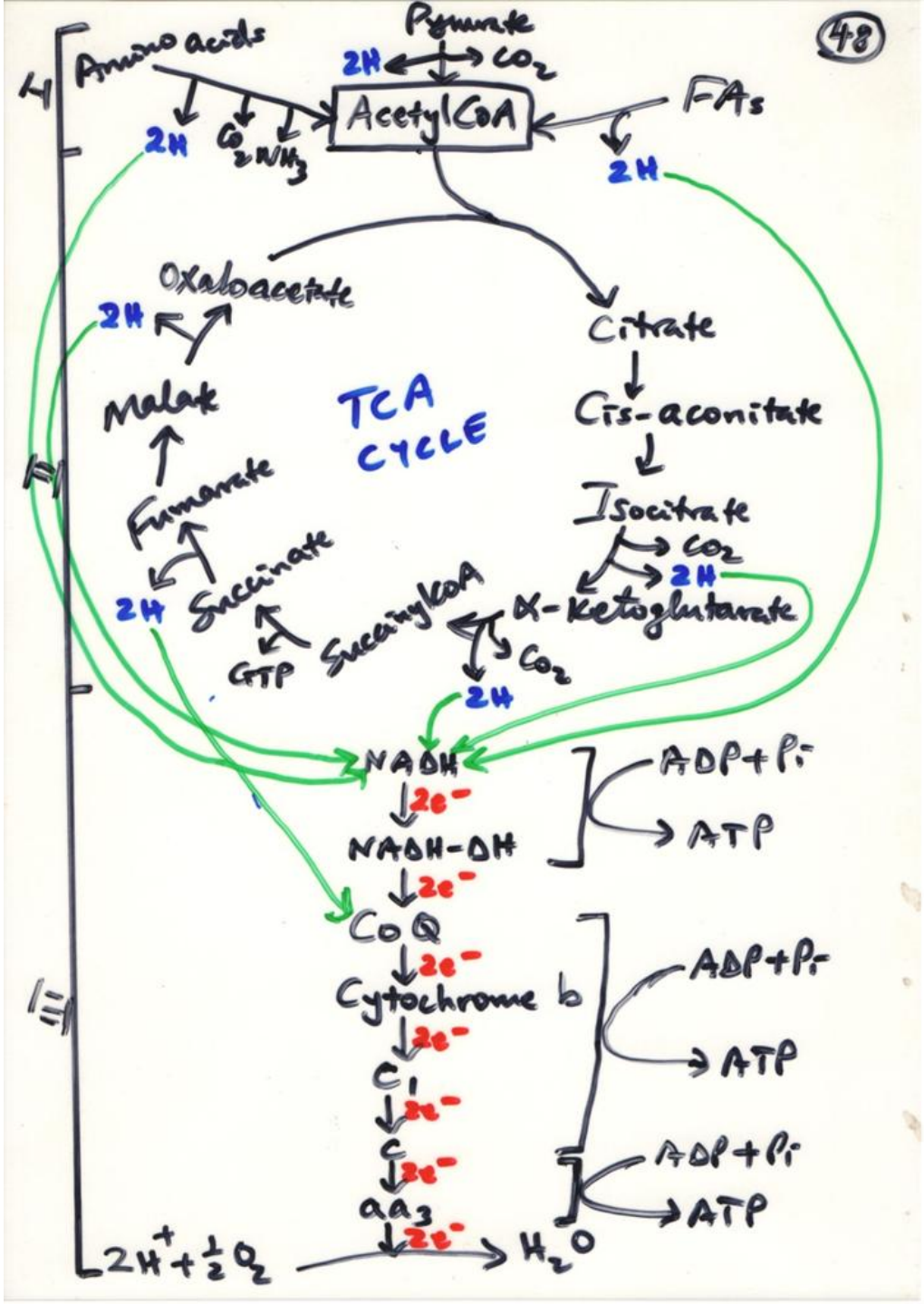
Mammals — Cells are aerobic so they oxidize organic fuels to $CO_2 + H_2O$. The aerobic phase of catabolism is known as respiration i.e. Consumption of O_2 and CO_2 formation by cells.

Respiration occurs in 3 major stages:

STAGE I — Organic fuels are oxidized to yield 2-Carbon groups in form of AcetylCoA.

STAGE II — The 2-Carbon groups (acetyl groups) to yield CO_2 and energy rich H_2 atoms.

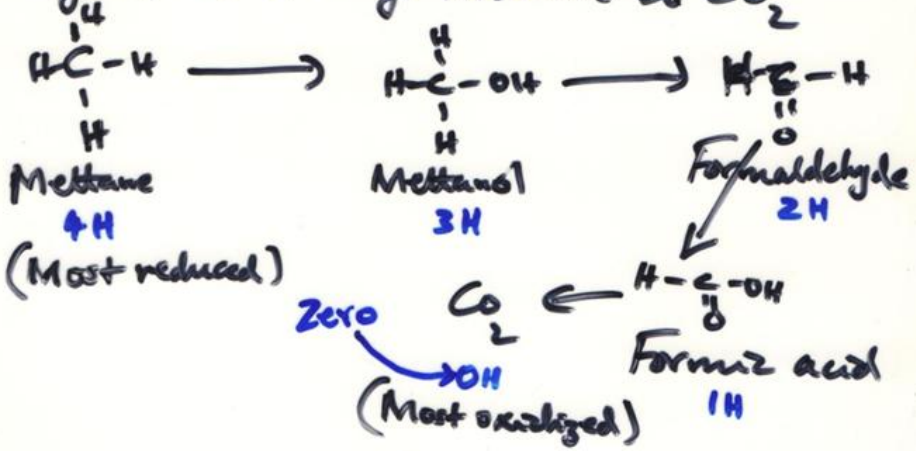
STAGE III — H atoms are split into $H^+ + e^-$. The e^- reduce O_2 to H_2O. ATP is formed.


Pyruvate
2H
CO_2
Amino acids
Acetyl CoA
FAs
2H
CO_2 NH_3
2H
Oxaloacetate
Citrate
2H
Malat
TCA
CYCLE
Cis-aconitate
Fumarate
Isocitrate
CO_2
2H
2H
Succinate
α-ketoglutarate
GTP
SuccinylCoA
CO_2
2H
NADH
ADP+Pi
2e⁻
ATP
NADH-DH
2e⁻
CoQ
2e⁻
Cytochrome b
ADP+Pi
2e⁻
ATP
c_1
2e⁻
c
2e⁻
ADP+Pi
aa_3
ATP
2e⁻
$2H^+ + \frac{1}{2}O_2$
H_2O

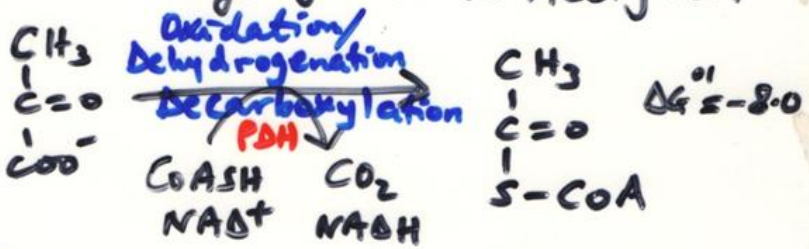
Glucose $\longrightarrow CO_2 + H_2O$ 100% energy released

Lactate – 93% energy

e.g. Oxidation of Methane to CO_2



e.g. Oxidation of Pyruvate to Acetyl CoA



Occurs in Mitochondria (matrix) in eucaryotes and cytoplasm in prokaryotes — In the presence of O_2.

PDH complex – 3 Enzymes ← E_1, E_2, E_3

+ 5 Coenzymes

TPP, FAD, $CoASH$, NAD^+, Lipoic acid

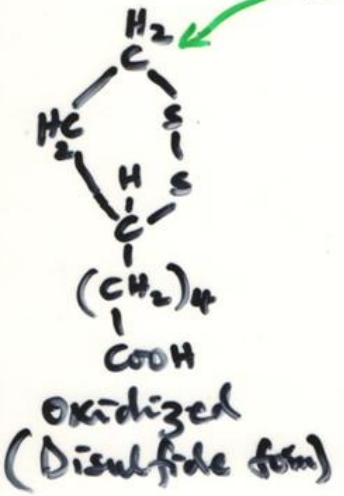
$E_1 - E_2 - E_3$

TPP → Transfers acetaldehyde group. "Active" aldehyde groups

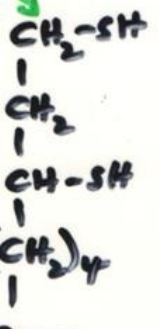
Lipoic acid (Core enzyme) → Transfers H atoms and acetyl groups from one enzyme to the other.

FAD → Transfers H atoms.

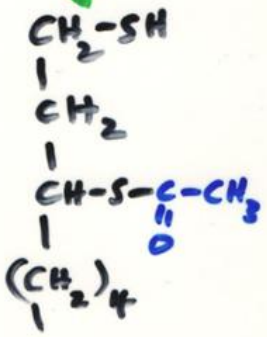
3 Forms



Oxidized (Disulfide form)



Reduced (Dithiol)



Acetylated form

Lipoic acid is bound to the enzyme (E_2) via a Lysine residue.

5 STEPS

1. $CH_3-\overset{O}{\overset{\|}{C}}-COO^- + H^+ + E_1\text{-}TPP \xrightarrow{E_1} E_1\text{-}TPP\text{-}CHOH\text{-}CH_3 + CO_2$

2. $E_1\text{-}TPP\text{-}CHOH\text{-}CH_3 + E_2$ (S–S) $\xrightarrow{E_1}$

$E_1\text{-}TPP +$ E_2 (S, SH) ← Thioester linkage → $CH_3-\overset{O}{\overset{\|}{C}}-$

3. E_2 (S, SH) $-\overset{O}{\overset{\|}{C}}-CH_3 + CoA\text{-}SH \xrightarrow{E_2}$

E_2 (SH, SH) $+ CoA\text{-}S-\overset{O}{\overset{\|}{C}}-CH_3$

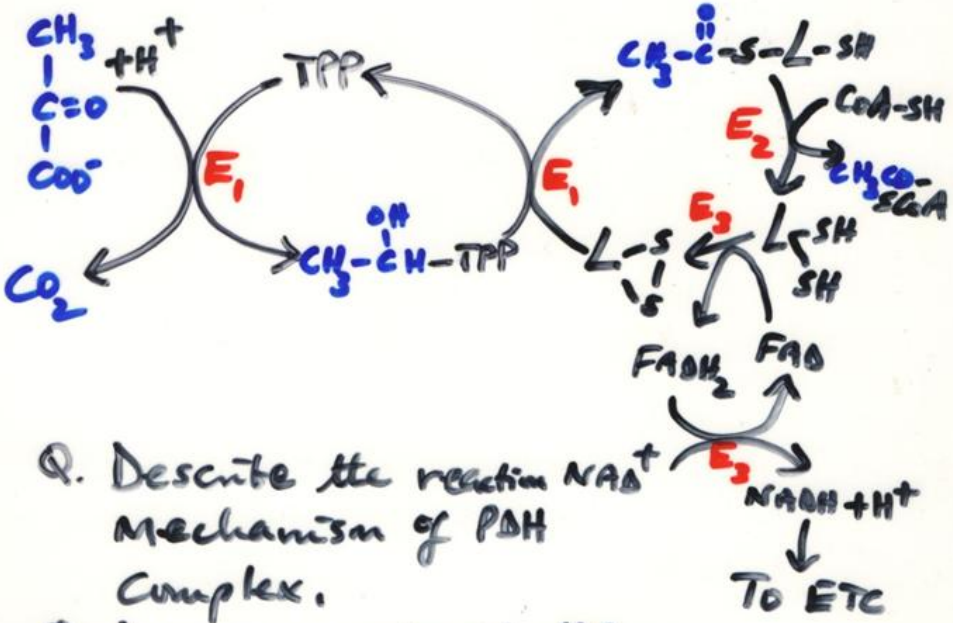
4. E_2 (SH, SH) $+ E_3\text{-}FAD \xrightarrow{E_3} E_2$ (S–S) $+ E_3\text{-}FADH_2$

5. $E_3\text{-}FADH_2 + NAD^+ \xrightarrow{E_3} E_3\text{-}FAD + NADH + H^+$

Sum:

$CH_3-\overset{O}{\overset{\|}{C}}-COO^- + CoA\text{-}SH + NAD^+ \longrightarrow CH_3-\overset{O}{\overset{\|}{C}}-S\text{-}CoA + CO_2 + NADH$

Reaction mechanism of PDH



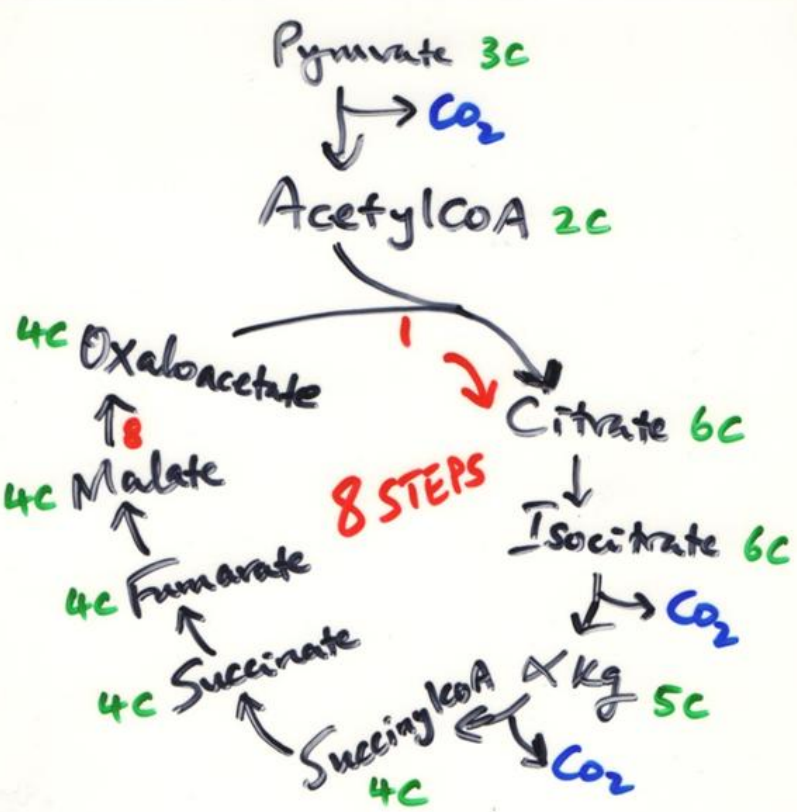
Q. Descute the reaction Mechanism of PDH Complex.

Q. Describe in detail, the Synthesis of acetylCoA from pyruvate.

Beriberi — Lack of Thiemine (Vit. B_1) — Pyruvate oxidation is impaired — in the brain — Polyneuritis

TCA CYCE.

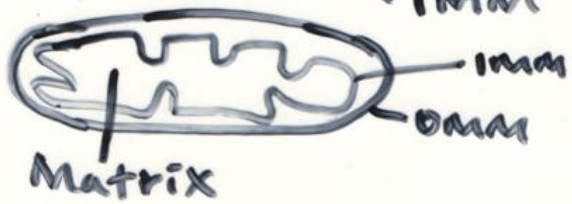
Linked to Pyruvate oxidation by acetylCoA.

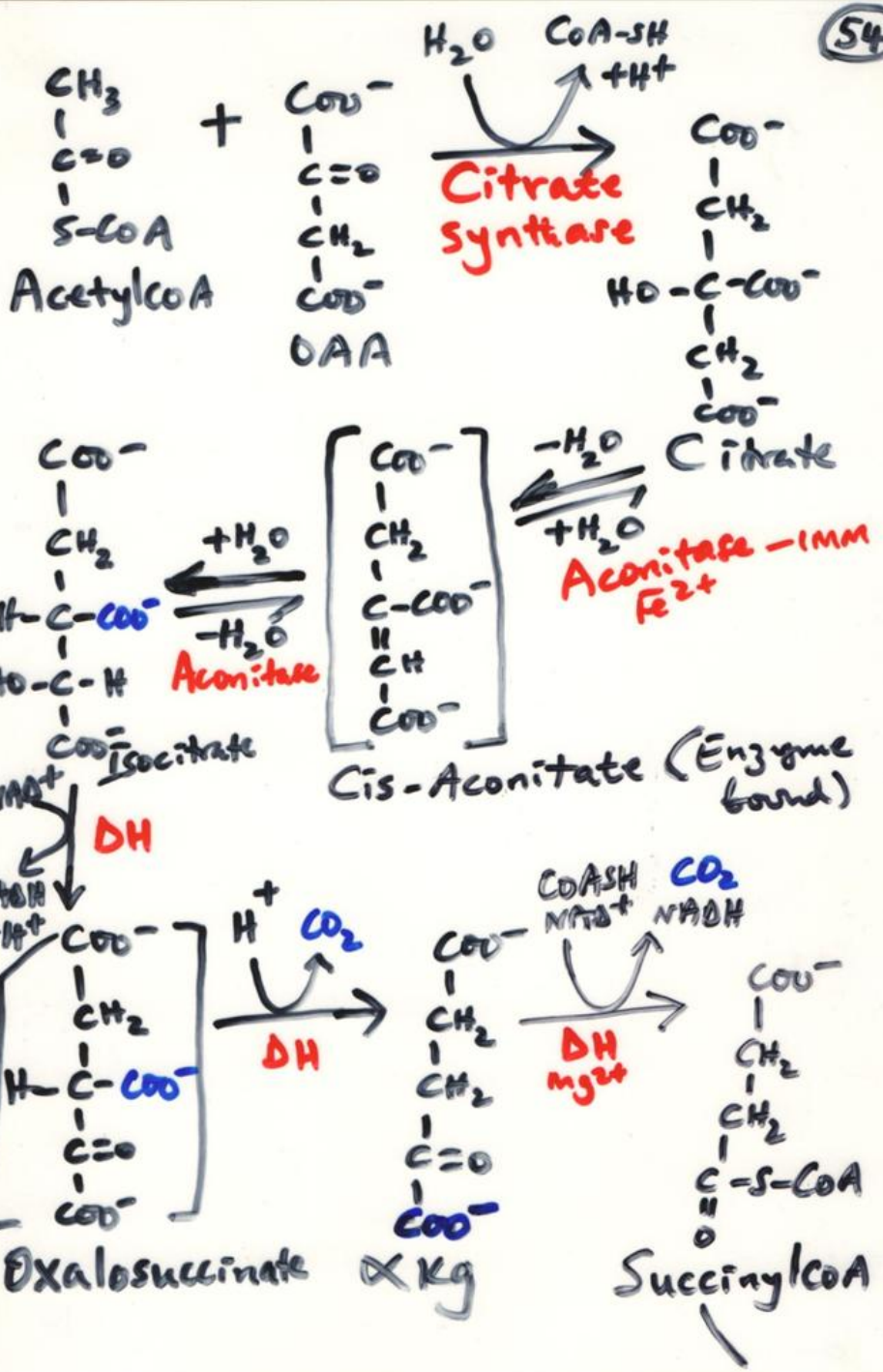


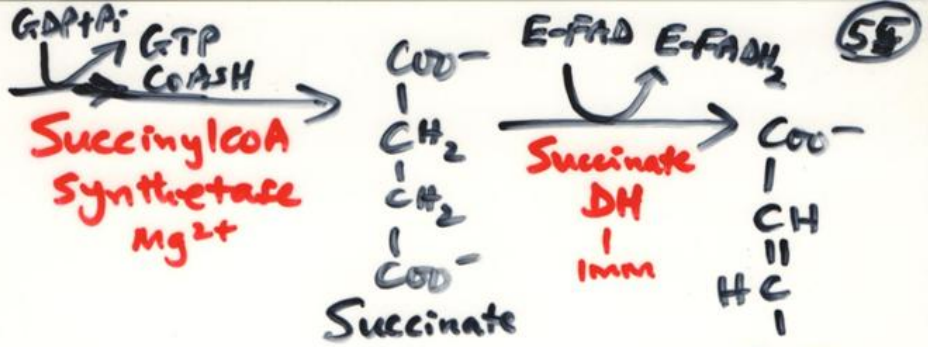
Each turn = 1 Acetyl group enters (2C)

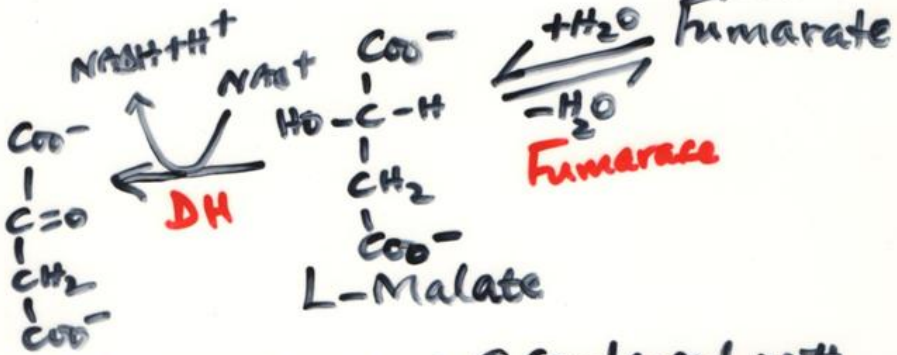
$2CO_2$ comes out.

The TCA cycle takes place in the Mitochondria < Matrix 6/8
IMM 2/8

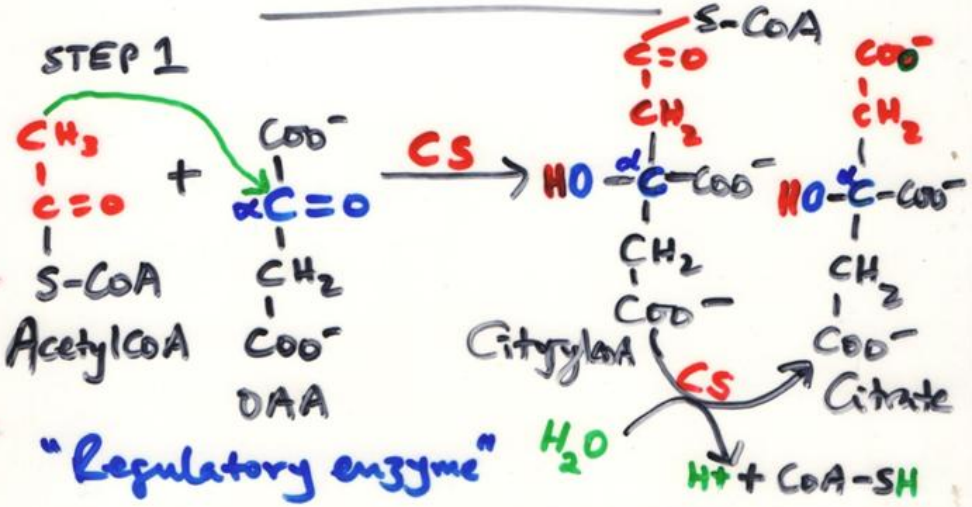



Acetyl CoA
OAA
Citrate synthase
Citrate
Aconitase
Aconitase – 1MM Fe2+
Isocitrate
Cis-Aconitate (Enzyme bound)
DH
Oxalosuccinate
αKg
Succinyl CoA

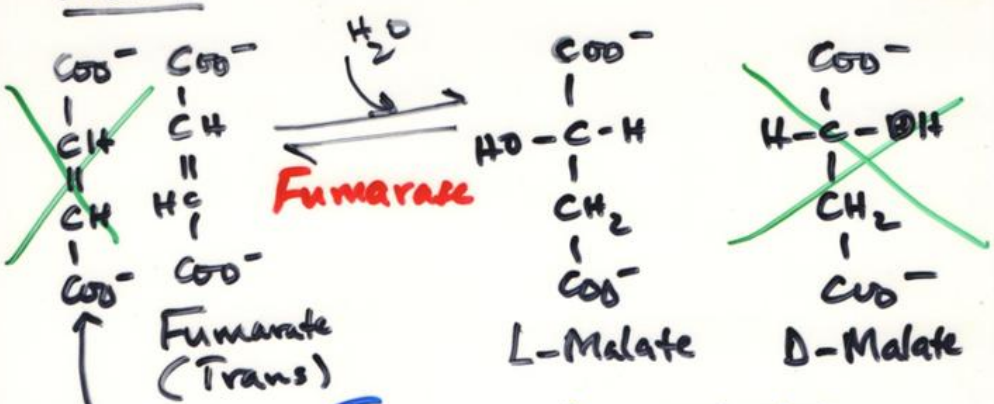




OAA → Recycled (Condensed with another molecule of Acetyl CoA).



STEP 7



Fumarase demonstrates specificity of enzymes towards their substrates.

Summary

4 pairs of H atoms generated;

- 3/4 reduce 3 molecules of NAD^+ to NADH
- 1/4 reduce 1 molecule of FAD to $FADH_2$

4 pairs of electrons pass the ETC to reduce 2 molecules of O_2 to $4H_2O$

i.e. $8H + 2O_2 \longrightarrow 4H_2O$ or

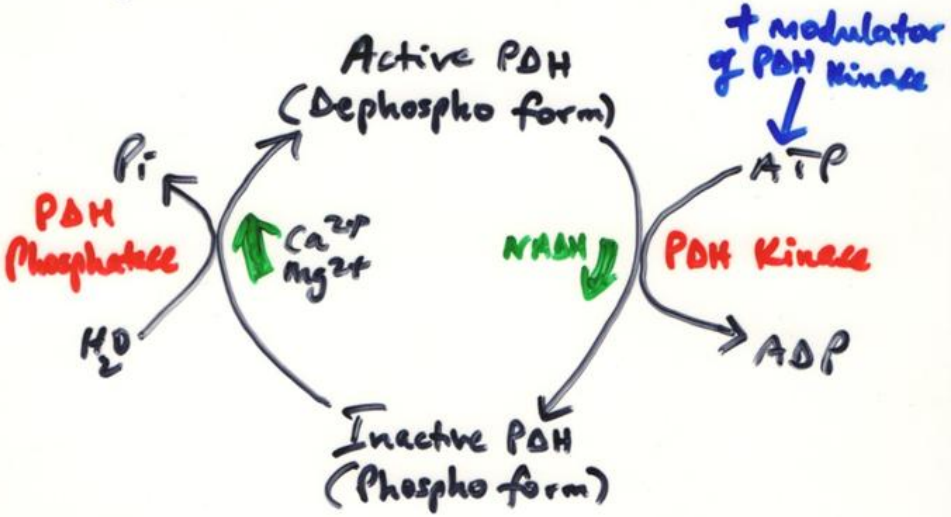
$8H^+ + 8e^- + 2O_2 \longrightarrow 4H_2O$ or

$* \; 2H^+ + 2e^- + \frac{1}{2}O_2 \longrightarrow H_2O$

Reduction of each atom of oxygen requires $2H^+ + 2e^-$

REGULATION OF PYRUVATE OXIDATION AND TCA CYCLE

1) Pyruvate oxidation



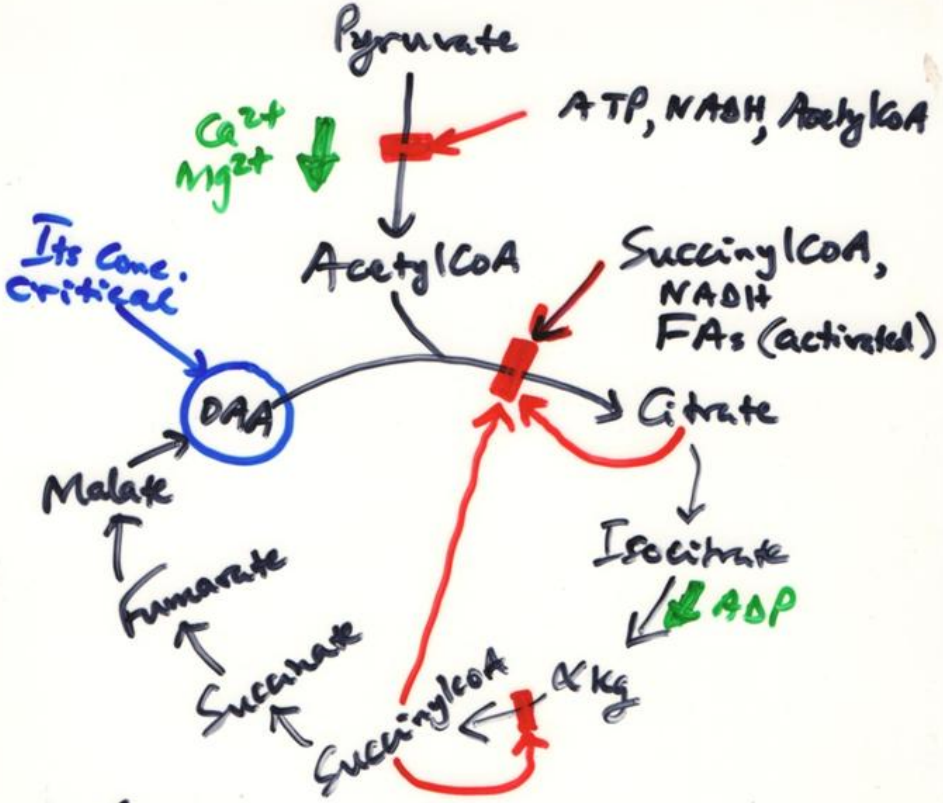
Covalent – by ATP acting on PDH kinase to inactivate.

Allosteric – [NADH]↑ [Acetyl CoA]↑ activates PDH kinase.

– FAs in form of fatty acyl CoA because they produce Acetyl CoA via β-oxidation.

– ↑[ATP] – deactivate PDH.

2) TCA cycle



∴ ↑[ATP], [NADH], [Citrate] inhibit glycolysis, pyruvate oxidation and TCA cycle. Their rates are integrated and matched to suit the needs of the cell.

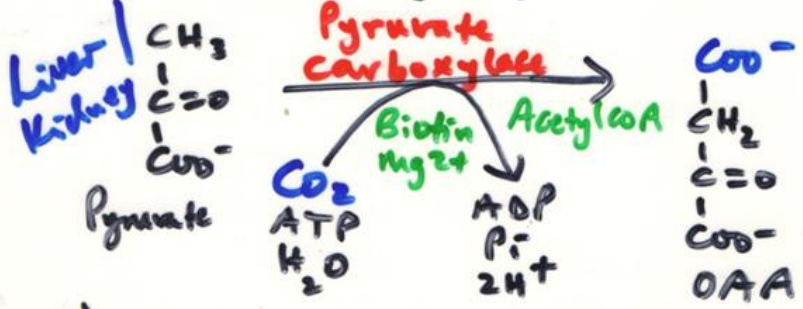
* – TCA cycle intermediates may be used for ~~other~~ metabolic purposes and they have to be replenished.

- The TCA cycle is an **dual** amphibolic pathway < Catabolic / Anabolic

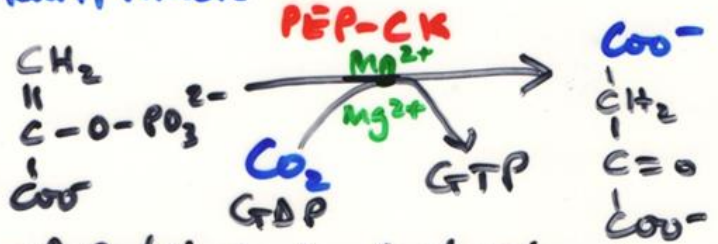
Intermediates e.g. αKg, OAA, Succinate can be removed from the cycle to make amino acids – their levels may go down – lowering the rate of the cycle.

* **Anaplerotic reactions** – replenish these intermediates;

1) Carboxylation of pyruvate to form OAA.



2) Carboxylation of PEP to form OAA



– A substrate-level phosphorylation

→ GTP + ADP ⇌ ATP + GDP

Other anaplerotic rxns;

3. Malic enzyme

Pyruvate ⟷ Malate → (TCA)

4. Glutamate dehydrogenase (GDH)

Glutamate ⇌ αKg → (TCA)

5. Glutamate oxaloacetate transaminase (GOT)

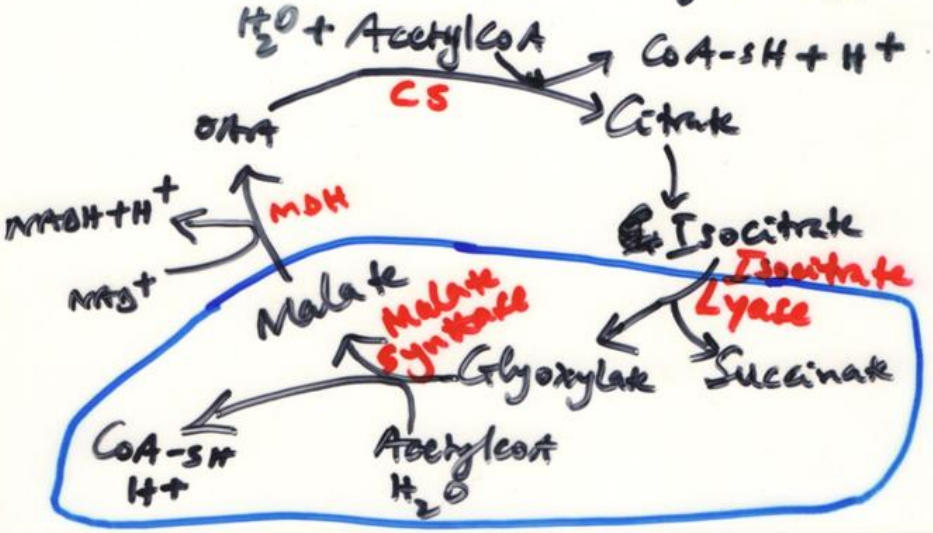
Glutamate + OAA ⇌ αKg + aspartate
→ TCA

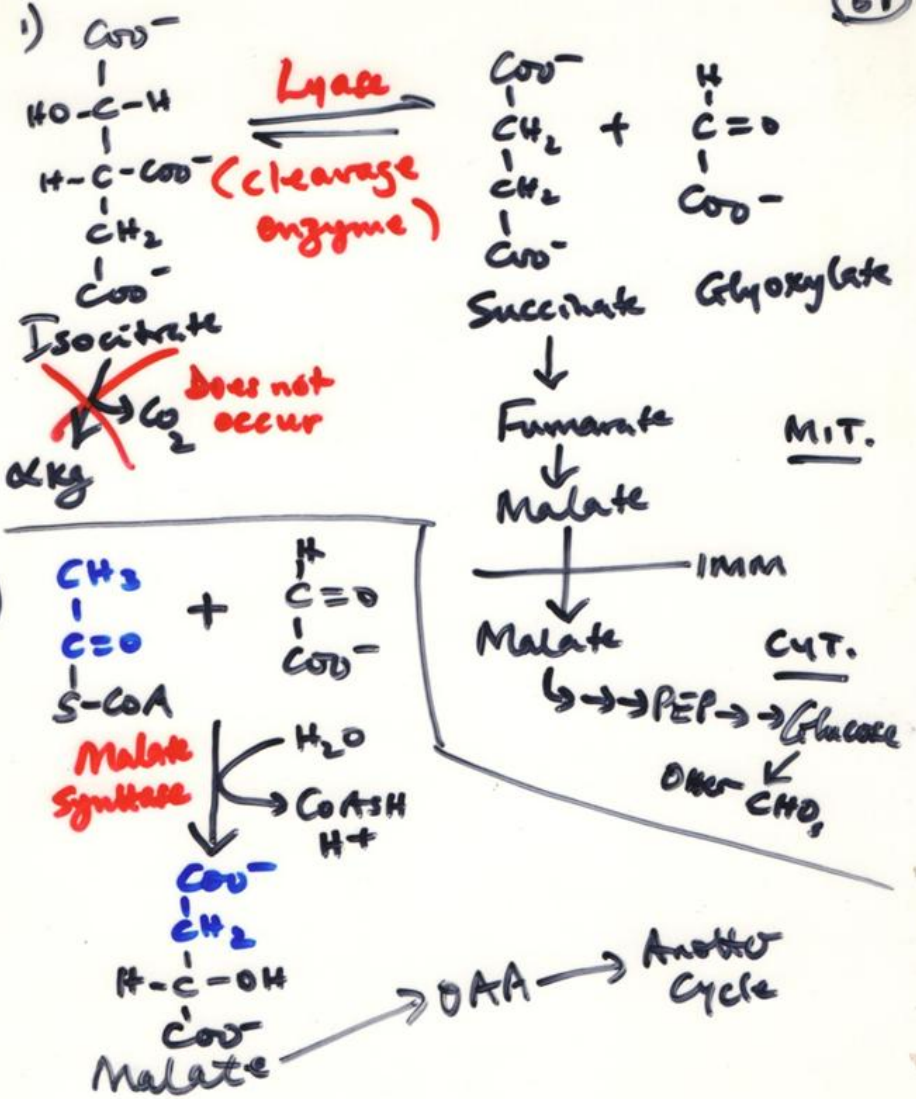
6. β oxidation of odd-chain FAs to succinyl CoA → Succinate → (TCA)

Others?

THE GLYOXYLATE CYCLE

- A modified form of the TCA cycle.
- Found in plants and some microorganisms such as E. Coli. In E. Coli, the acetyl groups (acetylCoA) may be used to provide energy i.e. $CO_2 + H_2O$ or can be used to synthesize CHOs. In E. Coli, TCA cycle can operate or it can be modified to a glyoxylate cycle.
- The glyoxylate cycle does not contain the decarboxylation reactions found in the TCA cycle. The CO_2 is needed in the synthesis of CHOs.





Plants — e.g. Germinating seeds

The cycle occurs in peroxisomes (Glyoxysomes) = Cytoplasmic organelles which act like mitochondria.

Glyoxysome – Contains the 2 enzymes

TGs —Lipolysis→ FAs —β-Oxidation→ AcetylCoA → Glyoxylate Cycle
↓
glycerol

Each turn of the cycle;
2 Molecules of acetylCoA enter (4C) and 1 molecule of succinate (4C) is formed and is used for biosynthetic purposes.

$2\ \text{AcetylCoA} + NH_4^+ + 2H_2O \longrightarrow$
$\text{Succinate} + 2\text{CoA} + NH_4OH + 3H^+$

ANIMALS – Lack the 2 enzymes – but they have gluconeogenic enzymes.

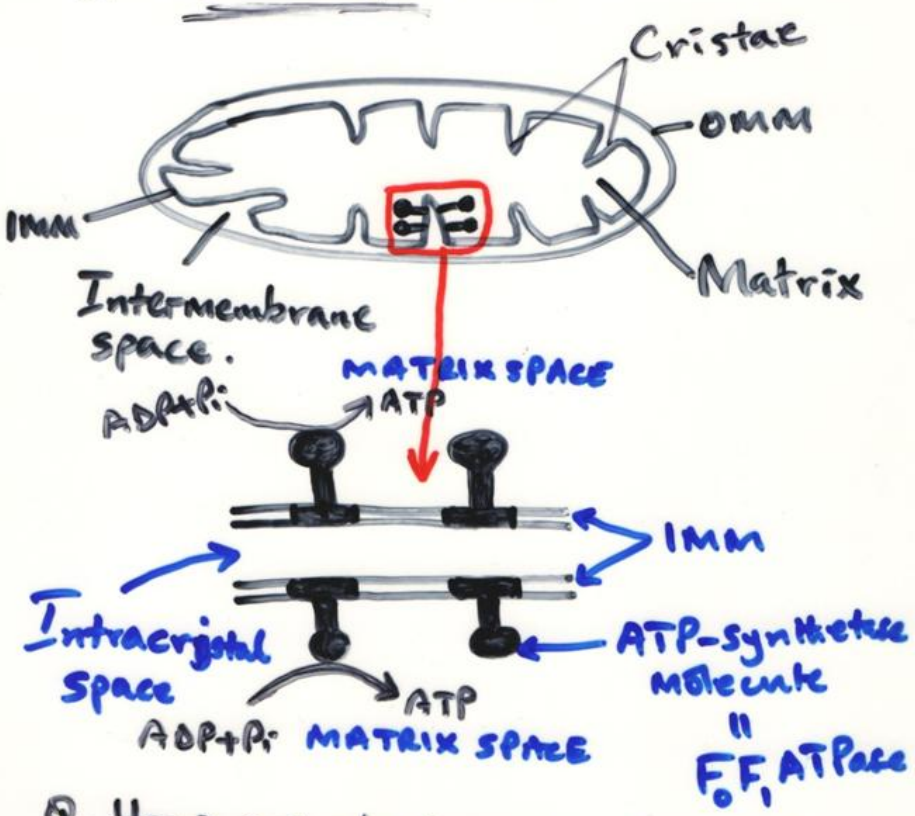
ELECTRON TRANSPORT / OXIDATIVE PHOSPHORYLATION. ENERGY TRANSDUCTION BY MITOCHONDRIAL MEMBRANES

Q. What is electron transport? It is the flow of electrons from organic substrates to molecular oxygen through a series of electron carriers that are located on the inner surface of the IMM. The flow yields energy for the synthesis of ATP.

Q. What is OP? It is the process in which ATP is formed as electrons are transferred from $NADH$ or $FADH_2$ to O_2 by a series of electron carriers.

∴ ET is coupled to OP.

THE BIOCHEMICAL ANATOMY OF THE MITOCHONDRIA



Q. How are electrons transferred?

Via REDOX reactions

The electron-donating molecule (carrier) and the electron-accepting molecule (carrier) are redox pairs. Reducing and oxidising agents function as conjugate reductant-oxidant pairs.

Proton donor $\rightleftharpoons$ H^+ + Proton acceptor

i.e. $\underset{(\text{Acid})}{HA} \rightleftharpoons H^+ + \underset{(\text{Conjugate base})}{A^-}$

Electron donor $\rightleftharpoons$ e^- + Electron acceptor

e.g. $Fe^{2+} \rightleftharpoons Fe^{3+} + e^-$

Redox pair

4 Ways – all occur in cells.

1) Transferred directly as electrons

e.g. Fe^{2+}–Fe^{3+} redox pair can transfer an electron to the Cu^+–Cu^{2+} pair.

$$\underset{(\text{red.})}{Fe^{2+}} + \underset{(\text{oxid.})}{Cu^{2+}} \longrightarrow \underset{(\text{oxid.})}{Fe^{3+}} + \underset{(\text{red.})}{Cu^+}$$

2) Transferred in form of hydrogen atoms

$$\underset{(\text{atom})}{H} \rightleftharpoons \underset{\text{Proton}}{H^+} + \underset{\text{electron}}{e^-}$$

$$AH_2 \rightleftharpoons A + 2H^+ + 2e^-$$

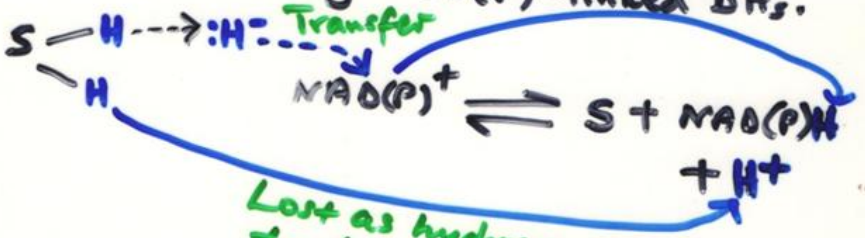
Hydrogen or electron donor

$AH_2 + B \longrightarrow A + BH_2$

Hydrogen donor — Hydrogen acceptor

- AH_2 and A are a conjugate redox pairs
- B and BH_2 are a conjugate redox pair.

3) Transferred from an electron donor to an acceptor in form of a hydride ion ($:H^-$) e.g NAD(P)-linked DHs.



4) Electron transfer may also occur when there is a direct combination of an organic reductant with O_2.

e.g. Oxidation of a hydrocarbon to an alcohol

Detoxification of drugs in Liver.

An electron participating in any redox reaction is called a reducing equivalent. In the mito., the reducing equivalents are in form of $:H^-$, H atoms or just e^- (electrons).

NB Each conjugate redox couple has a characteristic standard potential = E'_0 = Standard oxidation-reduction potential.

$HA \rightleftharpoons H^+ + A^-$ $K' = \dfrac{[H^+][A^-]}{[HA]}$

(Tendency of a conjugate acid-base pair to lose a proton is given by the dissociation constant.

∴ The tendency of a given conjugate redox pair to lose an electron is given by a constant = E'_0.

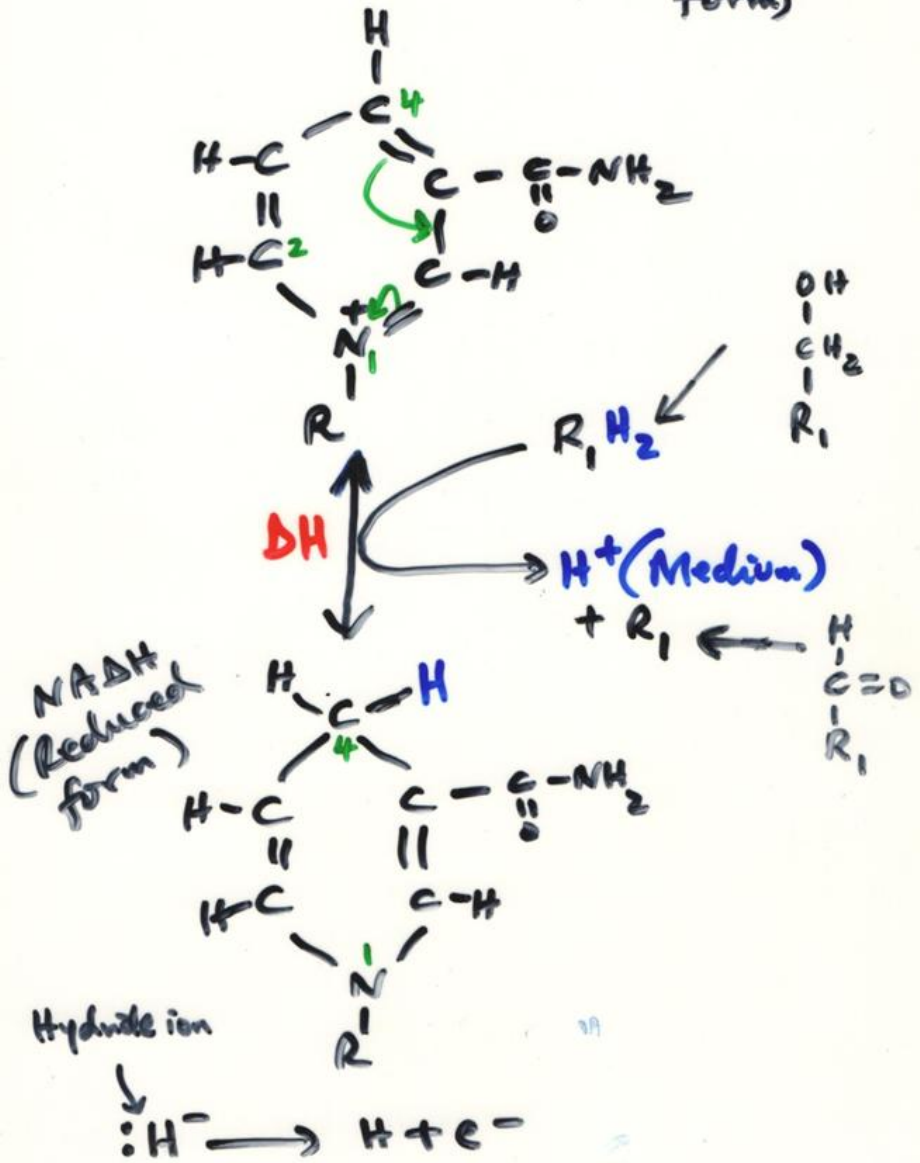
Definition: "The emf ----).

STD conditions = 1 M Conc.
25°C
pH 7.0

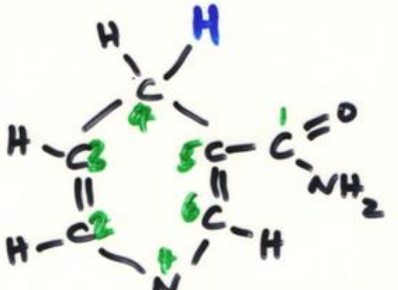
e.g. $H_2 \rightarrow 2H^+ + 2e^-$

Donor Acceptor $H_2/2H^+$ redox pair (couple).

NAD$^+$ = Nicotinamide Adenine Dinucleotide (oxidized form)



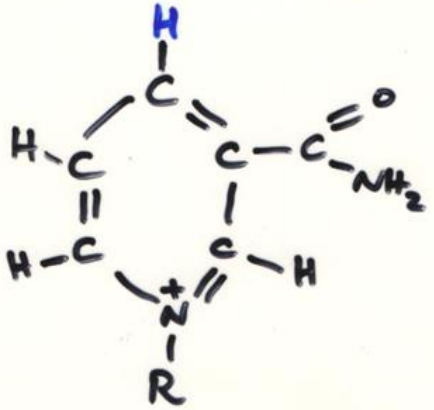
NADPH = Nicotinamide Adenine Dinucleotide Phosphate (Reduced form)

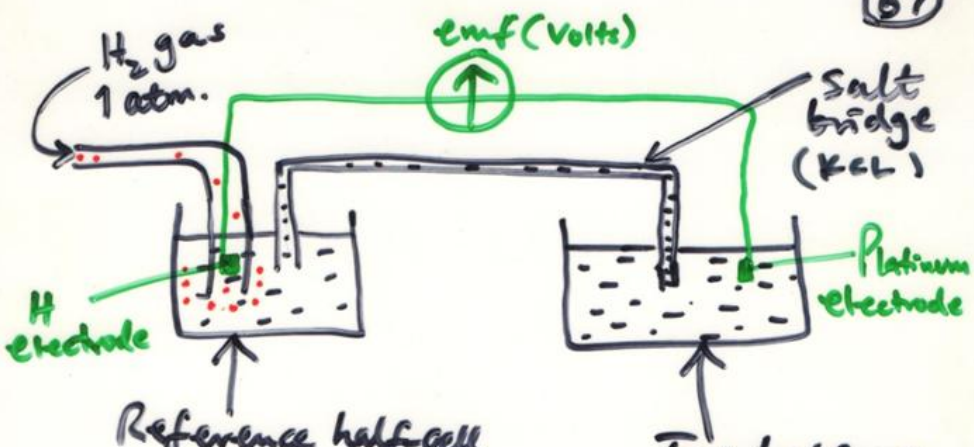


Nicotinamide ring

R = 1 Adenine base
3 Phosphate groups
2 Ribose sugars

$NADP^+$ = Oxidized form





Reference half-cell
of known emf = STD
H electrode where H_2 gas at 1 atm is equilibrated at the electrode with 1M H^+ to give emf of 0.0V (arbitrary). But

$H_2 \longrightarrow 2H^+ + 2e^-$

$H_2/2H^+$ couple at pH 7.0 = −0.41V

pH 0.0

Test half-cell
Containing 1M Concs. of the redox pair i.e. oxidized and reduced species to be tested.

E^1_o = − in systems having an increasing tendency to lose electrons.

= + ... gain electrons.

e.g. $\frac{1}{2}O_2/H_2O$ +0.82V

e.g. $H_2/2H^+$ −0.41V

E_0' of Conjugate redox couples participating in the ETC

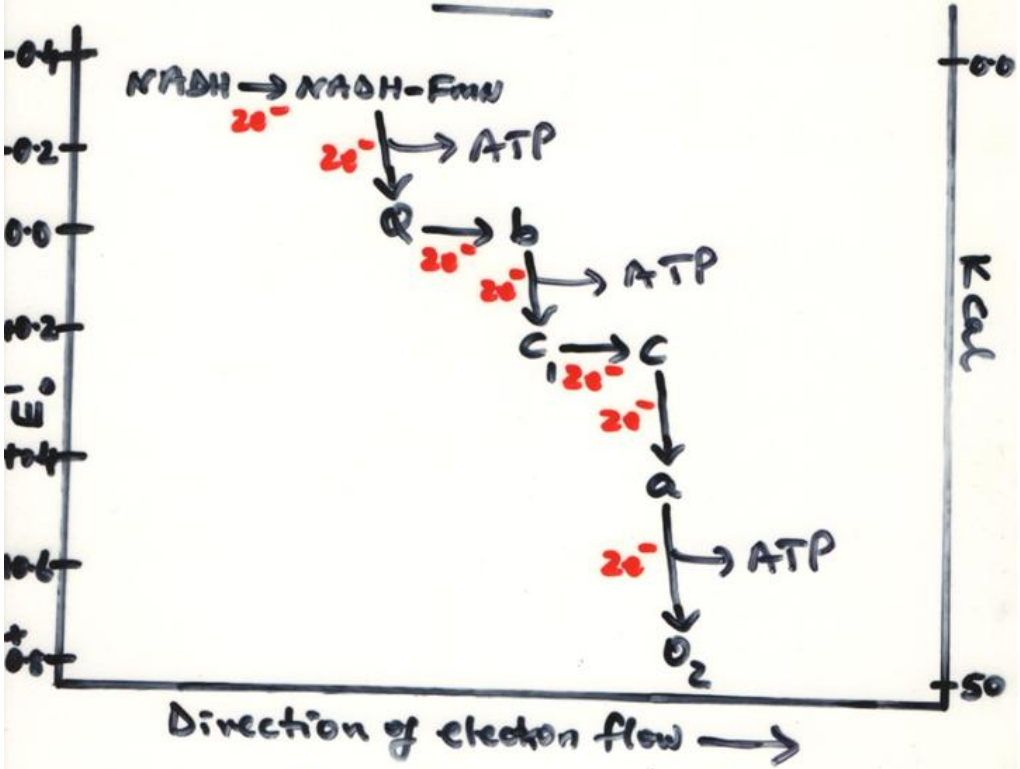
	E_0' (V)
1. $2H^+ + 2e^- \longrightarrow H_2$	−0.41
2. $NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.32
3. $NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.32
4. NADH-DH (FMN) $+ 2H^+ + 2e^- \longrightarrow$ NADH-DH ($FMNH_2$)	−0.30
5. Ubiquinone $+ 2H^+ + 2e^- \longrightarrow$ Ubiquinol	+0.04
6. Cyt. b (Oxid.) $+ e^- \longrightarrow$ Cyt. b (red.)	+0.07
7. Cyt. c_1 (Oxid.) $+ e^- \longrightarrow$ Cyt. c_1 (red.)	+0.23
8. Cyt. c (Oxid.) $+ e^- \longrightarrow$ Cyt. c (red.)	+0.25
9. Cyt. a (Oxid.) $+ e^- \longrightarrow$ Cyt. a (red.)	+0.29
10. Cyt. a_3 (Oxid.) $+ e^- \longrightarrow$ Cyt. a_3 (red.)	+0.55
11. $\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	+0.82

Increasing potential = in the order of decreasing tendency to lose electrons.

* In the order of increasing tendency to accept electrons.

4–5 6–7 9–11 ATP formed

Energy diagram of electron flow.



Electron flow from NADH to Mol. O_2

i.e. from -0.32 V to $+0.82$ V $\Rightarrow$ Loss of free energy

$$\Delta G'^{0} = -n F \Delta E'_{0}$$

- $\Delta G'^{0}$: STD free energy change in Calories
- n: no. of e⁻ transferred
- F: Faraday (23,062 Cal/V.mol) = CONSTANT
- $\Delta E'_{0}$: Change in E'_{0}

$\Delta G^{o'} = -2(23{,}062)[0.82-(-0.32)]$

$\simeq -52.6$ Kcal

$\simeq -53$ Kcal

The span of ETC is 1.14 V

This is the overall free energy change for the redox reaction at pH 7.0 when O_2, NAD^+, NADH and H_2O are all present at 1M concs.

a) $NAD^+ + H^+ + 2e^- \longrightarrow NADH$ $-0.32V$

b) $\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$ $+0.82V$

NET

$\frac{1}{2}O_2 + NADH + H^+ \longrightarrow H_2O + NAD^+ + 1.14V$

Q. How many ATPs are formed?

$3(7.3) = 21.9$ Kcal $= 3$ ATPs.

$\therefore 53$ Kcal $- 22.0$ Kcal $= 31$ Kcal

Lost in form of heat.

$\therefore$ Only about 42% of the energy generated by the electron flow is tapped in form of ATP. The rest is lost in form of heat. This is at STD conditions.

Q. Through which molecules do electrons pass?

Substrate $\xrightarrow{2e^-}$ O_2

> 15 Chemical groups (Proteins)
- Water insoluble
- Embedded in the IMM.

1. NAD – active in various DHs
2. FMN – active in NADH-DH
3. Coenzyme Q (Ubiquinone) – an Isoprenoid lipid-soluble quinone which functions with one or more proteins.
4. 2 kinds of Iron-Containing proteins
   (a) Iron-Sulfur centres (Fe-S)
   Non-heme proteins. They undergo Fe^{2+}-Fe^{3+} cycles.

$[Fe\text{-}S]_n \xrightarrow{2e^-} CoQ$

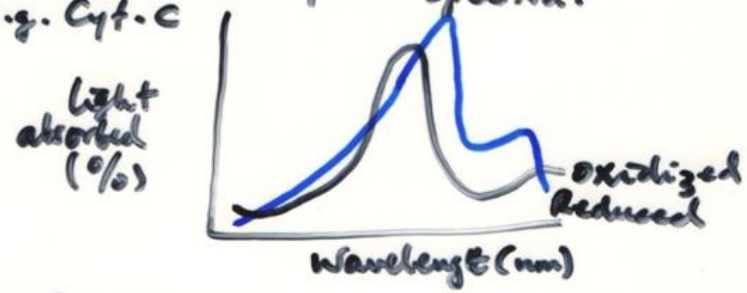
$Fe^{3+} + e^- \rightleftharpoons Fe^{2+}$

b) Cytochromes – Heme proteins

3 classes ← a – Heme A and Copper
b, c } Heme Porphyrin IX (9)

They are distinguished by differences in their light absorption spectra.

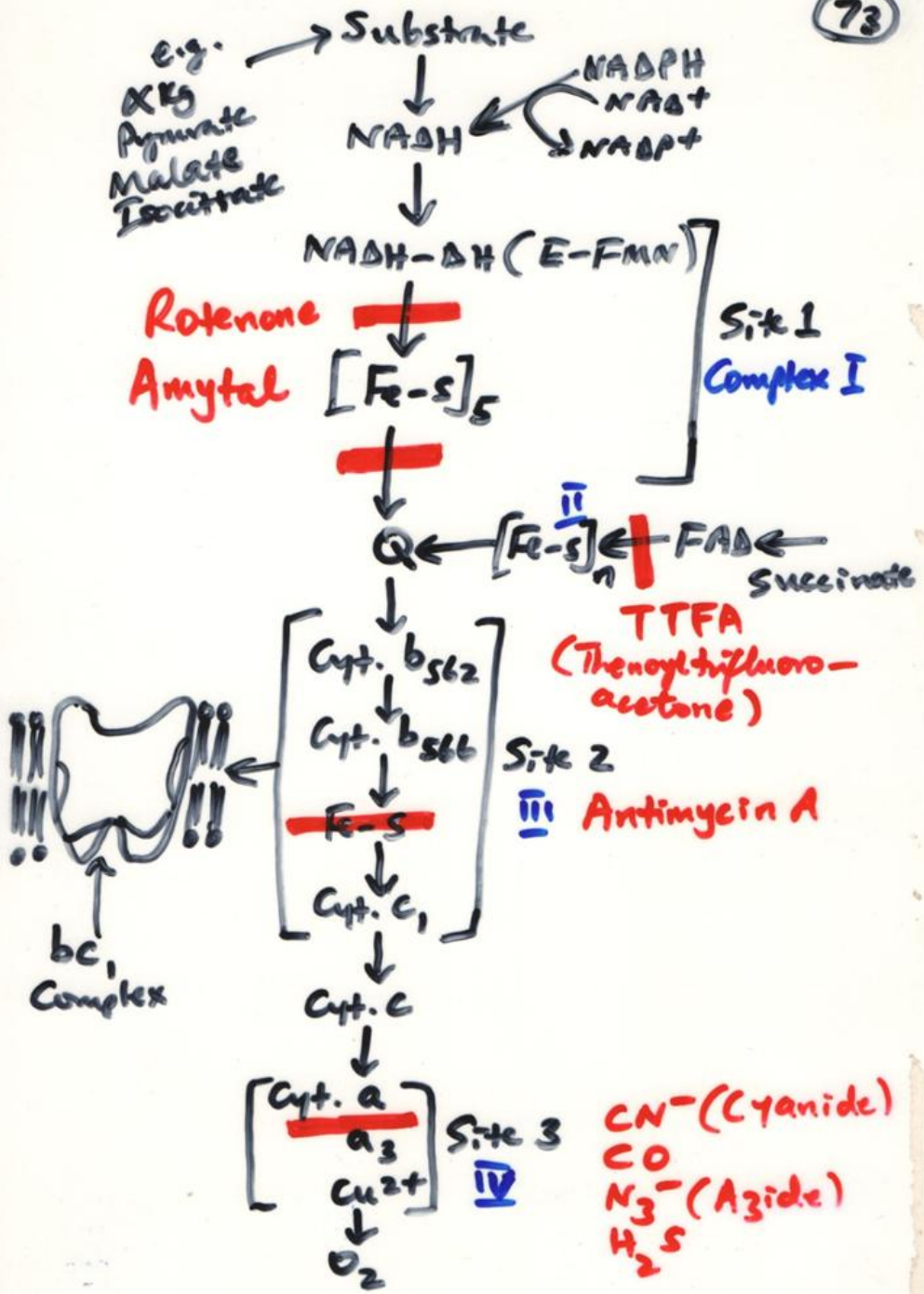
e.g. Cyt. c



- Cytochromes are reddish-brown proteins.
- Carry electrons from Q to O_2.

The ETC consists of about 4 Protein complexes that can be Isolated as functional assemblies.

1. Complex I (Site 1) = NADH – CoQ reductase.
2. II Succinate – CoQ reductase
3. III (Site 2) = $CoQH_2$ – Cyt. c reductase (Cytochrome reductase)
4. IV (Site 3) = Cyt. c – Cyt. a oxidase (Cytochrome oxidase)


e.g.
αKG
Pyruvate
Malate
Isocitrate
Substrate
NADH
NADPH
NADP+
NADH-DH (E-FMN)
Rotenone
Amytal
[Fe-S]
Site 1
Complex I
Q
[Fe-S]
FAD
Succinate
TTFA
(Thenoyltrifluoro-
acetone)
Cyt. b562
Cyt. b566
Site 2
III Antimycin A
Fe-S
Cyt. c1
bc1
Complex
Cyt. c
Cyt. a
a3
Cu2+
O2
Site 3
IV
CN- (Cyanide)
CO
N3- (Azide)
H2S

INHIBITORS OF ET and OP

1. Inhibitors of Complex I
   - Rotenone - natural product
   - Amytal - Barbiturate
   - Demerol - A painkiller

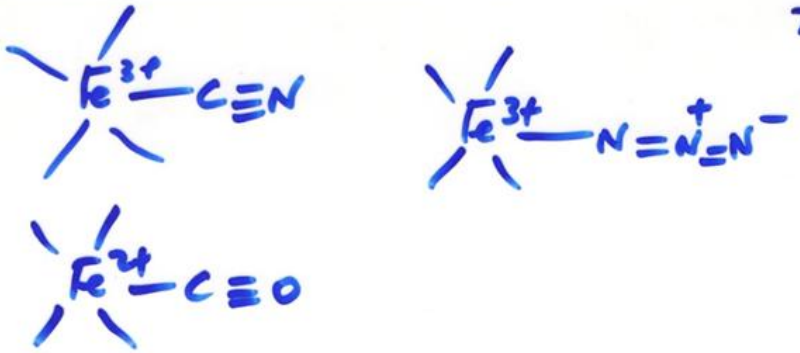
   * They inhibit or block the oxidation of Fe-S clusters of Complex I.

2. Inhibitors of complex II
   - TTFA
   - Carboxin

3. Inhibitors of complex III
   - Antimycin A

4. Inhibitors of Complex IV
   - $-C\equiv N$ Cyanide
   - $-N=N^{+}=N^{-}$ Azide
   - $C\equiv O$ Carbon monoxide

   * They bind to the heme of Cytochrome oxidase.
   * They bind to Fe^{2+}/Fe^{3+} in heme.



5. Inhibitors of ATP-synthetase (OP)

- Oligomycin – binds to F_o subunit blocking the flow of protons through the channel.
- Dicyclohexylcarbodiimide (DCCD) (forms covalent bonds to glutamate residues of F_o – blocking the H^+ channel.

~~[illegible]~~

- DNP
- Ionophores e.g. Valinomycin
- Dicumarol
- FCCP

* – Thermogenin – UCP1

Q. How is the ATP synthesized?

By an ATP-synthesizing enzyme located in the IMM. It is a complex consisting of 2 major components — F_0 and F_1 (Knob)

↑ Stalk

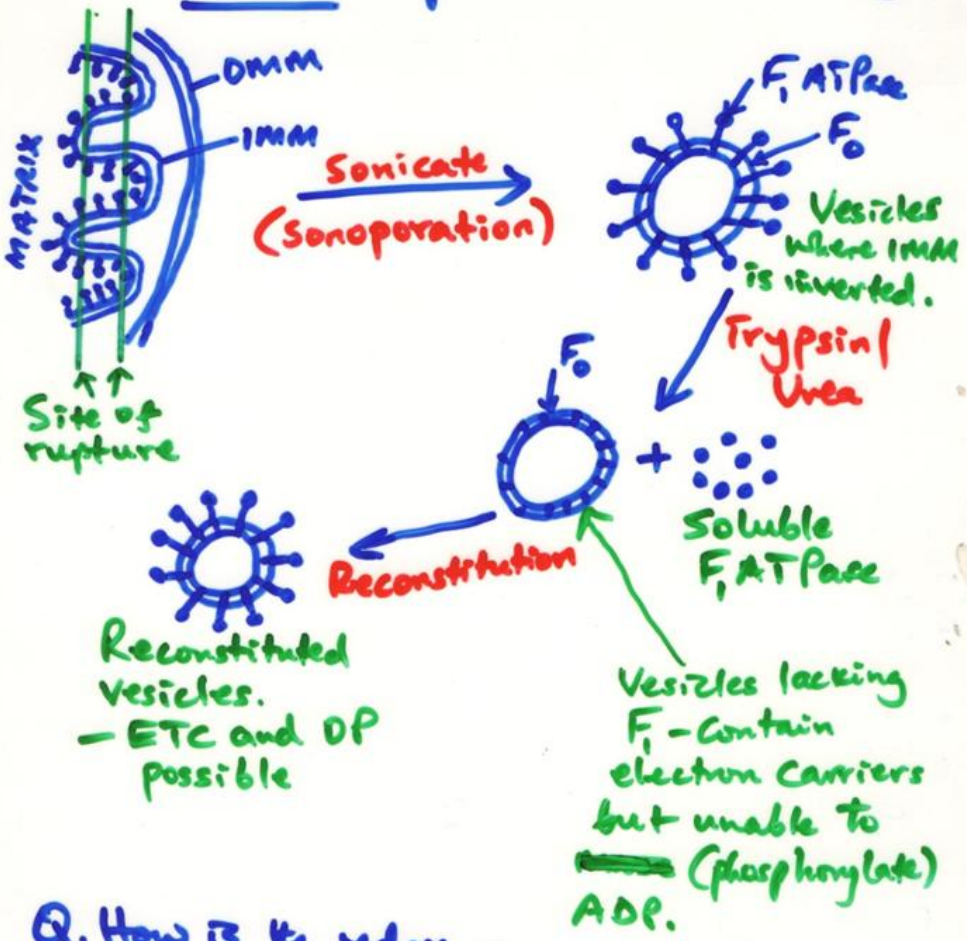
F_0 - Located on both sides of the IMM.
- Contains the electron carriers.
- Insoluble.

F_1 - Also called F_1 ATPase because it can hydrolyze ATP but cannot make ATP. When bound to F_0, it can synthesize ATP.
- 9 chains arranged in a cluster.
- Has binding sites for ATP.
- Soluble

F_1 = 380,000 Mwt. Daltons

Q. What exp. led to the understanding the role of both F_0 and F_1?

Sonication exp.



Q. How is the redox energy of electron transport delivered to ATP-synthetase?

Q. How does the ETC cooperate with the ATP synthetase to bring about OP of ADP to ATP?

3 mechanisms proposed = Hypotheses

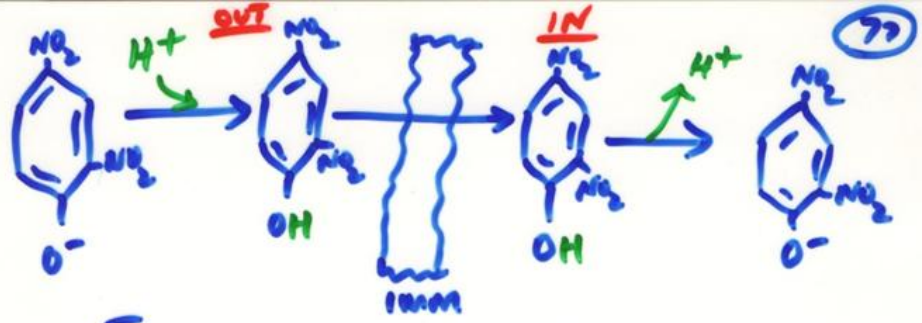
1. The chemical coupling hypothesis.

2. The conformational-coupling hypothesis.
3. The chemiosmotic hypothesis.

Q. What are the characteristic properties of oxidative phosphorylation that support the chemiosmotic hypothesis?

Q. What are the mitochondrial properties that support the chemiosmotic hypothesis?

1. No "high energy" intermediates linking ET to ATP synthesis has been found.
2. OP requires intact IMM structure i.e. Sonication exp.
3. The IMM is impermeable to H^+, OH^-, K^+ and Cl^- ions. If the membrane is damaged, OP does not occur.
   ∴ A difference in ionic composition or conc. across the IMM is essential for ATP synthesis.
4. OP can be prevented by uncoupling agents called protonophores e.g. 2, 4-Dinitrophenol. It is an uncoupler of OP. ET occurs but no OP occurs.



- The uncoupler binds H^+ and transports it to a medium of lower conc. thereby preventing the formation of a H^+ gradient across the imm.
- The uncoupler delinks the two systems and the energy produced by ET appears as heat but not as ATP. i.e. ET occurs but no OP!

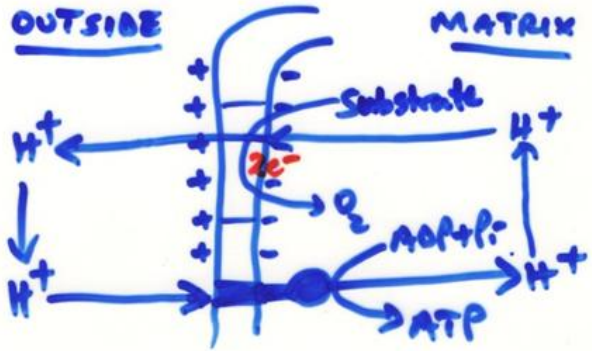
5. OP can be prevented by ionophores. These are ion carriers.
   e.g. Valinomycin (toxic antibiotic) transports K^+
   e.g. Gramicidin – K^+ and Na^+

∴ Increasing permeability of the imm to H^+, K^+, Na^+ etc by ionophores prevents OP. The proton gradient across the membrane is destroyed.
∴ The integrity of the imm must be maintained. "Intact".

Q. What does the Chemiosmotic hypothesis propose?

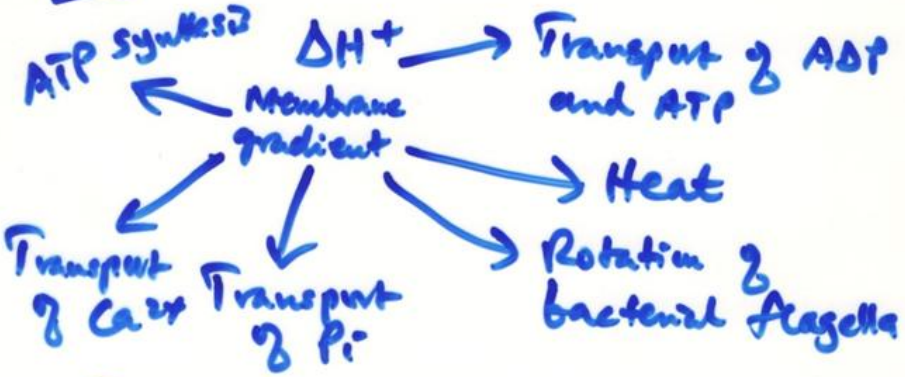
1. A proton gradient carries energy from ET to ATP synthesis.
2. The ET pumps H^+ from the matrix to the outer medium - generating an acid outside gradient of H^+ between the 2 aqueous phases separated by the IMM.

3. Such a H^+ gradient contains potential (osmotic) energy = pH gradient.
4. Protons flow back to matrix via the F_0F_1 ATPase.

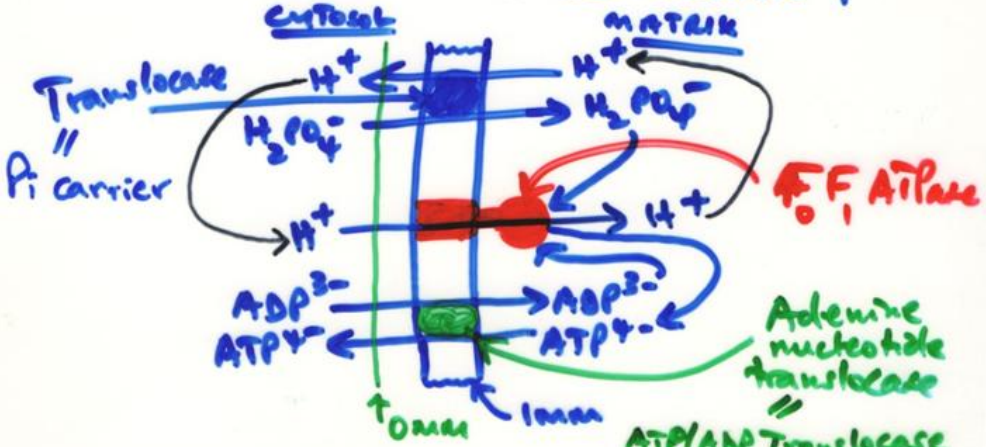


Membrane gradient / Proton gradient / Electrochemical gradient / Osmotic gradient

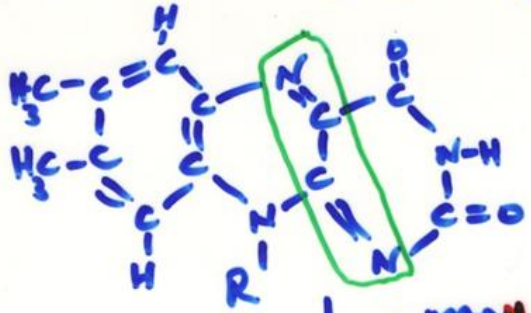
Uses.



Q. The inner is impermeable not only to H^+, OH^- and K^+ but also to many other ionic solutes. How, then is ADP and Pi formed in the cytosol enter the matrix and how does ATP leave the matrix?

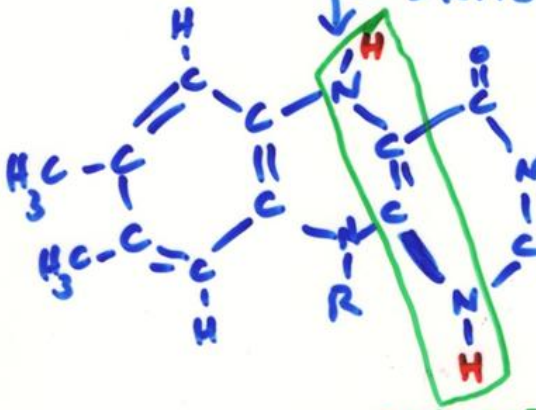


$NADH + H^+ + E\text{-}FMN \rightleftharpoons NAD^+ + E\text{-}FMNH_2$



oxidized (E-FMN)

$NADH + H^+ \rightarrow NAD^+$



Reduced ($E\text{-}FMNH_2$)

$E\text{-}FMNH_2 + Q \overset{2H^+ + 2e^-}{\rightleftharpoons} E\text{-}FMN + QH_2$

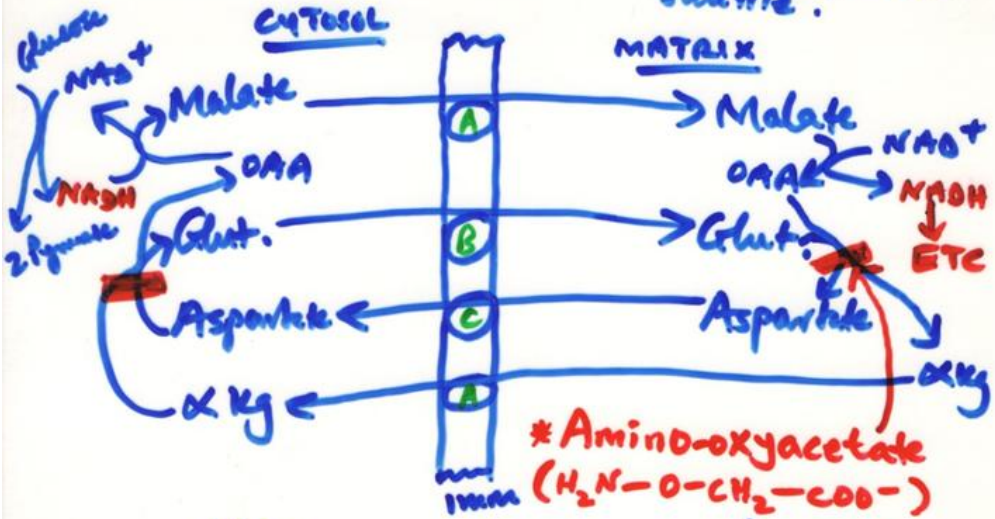
$QH_2 + \underset{(oxid.)}{Cyt.b} \overset{2H^+ + 2e^-}{\rightleftharpoons} Q + Cyt.b\,(red.)$

SHUTTLE SYSTEMS

The NADH-DH of the IMM accept electrons only from Mit. NADH

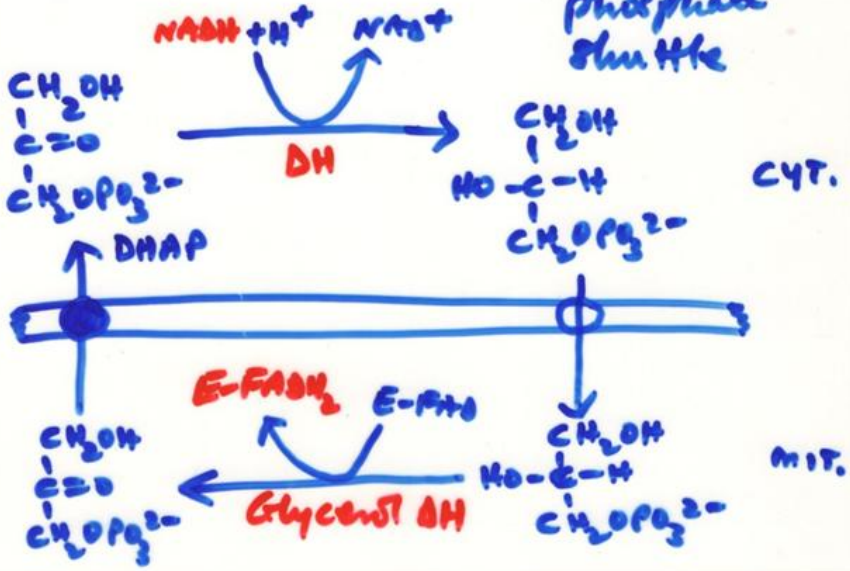
Q. How is cytosolic NADH able to cross the IMM?

1. Liver / Heart / Kidney – Malate-Aspartate Shuttle



$$NADH_{(Cyt.)} + NAD^+_{(Mit.)} \rightleftharpoons NAD^+_{(Cyt.)} + NADH_{(Mit.)}$$

2. Skeletal Muscle / Brain – Glycerol-phosphate Shuttle



GPS – $NADH_{(Cyt.)} + H^+ + E\text{-}FAD_{(Mit.)} \longrightarrow NAD^+_{(Cyt.)} + E\text{-}FADH_{2\,(Mit.)}$

MAS – $NADH_{(Cyt.)} + NAD^+_{(Mit.)} \rightleftharpoons NAD^+_{(Cyt.)} + NADH_{(Mit.)}$

Q. How many ATPs are formed from complete oxidation of glucose to CO_2 and H_2O?

Q. Account for the number of ATPs formed (made) from complete oxidation of a glucose molecule.

1. 1 Glucose $\xrightarrow[\text{(Partial oxidation)}]{\text{Glycolysis}}$ 2 Pyruvate
(CYTOSOLIC)

2 ATP

$Glucose + 2P_i + 2ADP + 2NAD^+ \longrightarrow$
$2\ Pyruvate + 2ATP + 2NADH + 2H^+ + 2H^+ + 2H_2O$

The 2NADH generated by the glycolytic pathway are carried into the Mit. by the Malate–Aspartate shuttle – enter the ETC and flow to O_2.

$2NADH + 2H^+ + 6P_i + 6ADP + O_2 \longrightarrow$
$2NAD^+ + 6ATP + 8H_2O$
$2(3ATPs) = 6ATP$ **6 ATP**

But if the glycerol-phosphate shuttle is used, the 2NADH enter the ETC as $FADH_2$ to O_2.

$2NADH + 2H^+ + FAD + 4P_i + 4ADP + O_2 \longrightarrow$
$2NAD^+ + 4ATP + H_2O + FADH_2$
$2(2ATPs) = 4ATP$ **4 ATP**

2. Dehydrogenation of 2 Pyruvate to 2 AcetylCoA and $2CO_2$ by the PDH complex, i.e. **6 ATP**

$2Pyruvate + 2CoA + 6P_i + 6ADP + O_2 \longrightarrow$
$2AcetylCoA + 2CO_2 + 6ATP + 8H_2O$

3. Oxidation of 2 AcetylCoA to $CO_2 + H_2O$. (TCA cycle)

$2AcetylCoA + 24P_i + 24ADP + 4O_2 \longrightarrow$
$2CoA\text{-}SH + 4CO_2 + 24ATP + 26H_2O$

What stages (steps)?

(a) 3 NADH from ICDH, α-KgDH and MDH
$3(3ATPs) = 9\ ATP \times 2 = 18\ ATP$

(b) 1 $FADH_2$ from SDH which passes through CoQ. 2 (2 ATPs) = 4 ATP / AcetylCoA

= 4 ATP

(c) 1 GTP arising from SuccinylCoA synthetase reaction / AcetylCoA

= 2 GTP

The 2 GTPs are converted to 2 ATPs

$GTP + ADP \rightleftharpoons GDP + ATP$

Total = 18 + 4 + 2 = 24 ATP

24 ATP

Grand total = 2 + 6 + 6 + 24 = 38 ATPs

38 ATP = by MAS

Grand total = 2 + 6 + 4 + 24 = 36 ATPs

36 ATP by GPs

∴ Glycolysis + Respiration

$Glucose + 38Pi + 38ADP + 6O_2 \longrightarrow 6CO_2 + 38ATP + 44H_2O$

The oxidation of glucose under STD conditions yields − 686 kcal

i.e. $\Delta G^{o'} = -686$ kcal

$Glucose + 6O_2 \longrightarrow 6CO_2 + 6H_2O$

$ATP + H_2O \rightarrow ADP + P_i \quad \Delta G^{\circ\prime} = -7.3$ kcal

$\therefore 38 \times -7.3 = -277.4$ kcal

Overall efficiency (%) $= \frac{277.4}{686} \times 100$

$\simeq 40\%$ under STD conditions.

Intact cell:

> 70% because [glucose], $[O_2]$, $[P_i]$, [ADP] and [ATP] are unequal and much lower than the concs. of 1.0 M assumed in STD free energy Calculations.

⇒ Homework

Calculate for F6P (39), AcetylCoA (12), G3P (20), Sucrose (76), Lactose (77?).

There are secondary pathways of glucose catabolism;

1) The pentose phosphate pathway (PPP).
2) The conversion of glucose to glucuronic acid and ascorbic acid.