Quantitative Metabolism

Consider typical biochemical pathways as follows :

Carbon Source

Energy Source

End Product Formation

Energy Formation (DIRECT)

Energy Formation (INDIRECT)

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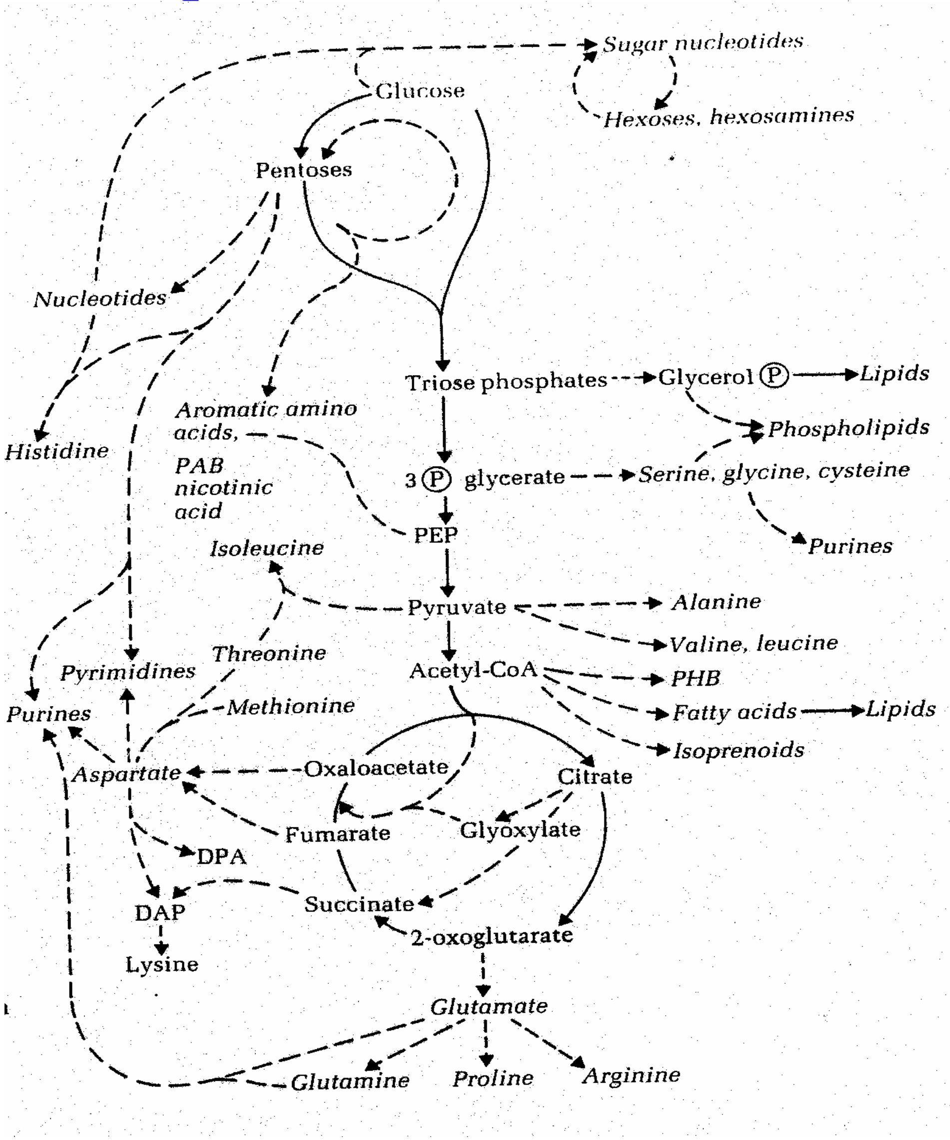
Quantitative Metabolism



Quantitative Metabolism

FULL LINES = Carbon Flow by CATABOLISM

BROKEN LINES = Carbon Flow by ANABOLISM

There are VERY MANY carbon skeletons servicing a large number of cell components from AMINO ACIDS (e.g. glutamate, proline, arginine etc) to LIPIDS to NUCLEOTIDES (for DNA / RNA) to POLYMERS of sugars (for CELL WALLS) etc.

Although we don't have the time to study ANABOLISM in detail, it is important to appreciate the origins of the biosynthetic components.

Quantitative Metabolism

The CATABOLISM of glucose AEROBICALLY may be broken down into three steps :

GLUCOSE	→	**PYRUVATE**	**(Glycolysis)**
PYRUVATE	→	**ACETYL CoA**	
ACETYL CoA	→	**CO_2 + H_2O**	**(TCA Cycle)**

Let us look at each of these processes briefly to identify :

Carbon Source

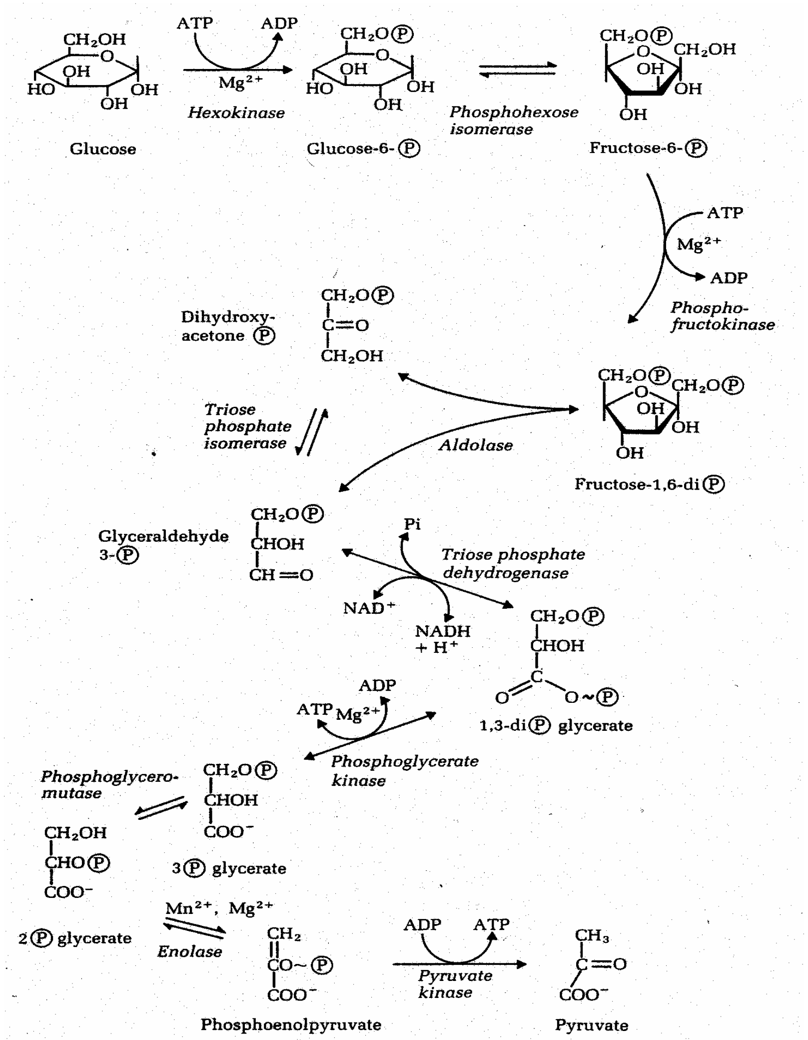
Energy Source

End Product(s)

Energy Formation (DIRECT)

Energy Formation (INDIRECT)

Quantitative Metabolism



Embden–Meyerhof–Parnas scheme for glucose fermentation.

Quantitative Metabolism

Carbon Source	**=**	**GLUCOSE**
Energy Source	**=**	**GLUCOSE**
End Product(s)	**=**	**2 x PYRUVATE**
Energy Formation (DIRECT)	**=**	**2 ATP (UTILISED)** **4 ATP (GENERATED)**
	=	***<u>NETT 2 ATP GENERATED</u>***
Energy Formation (INDIRECT)	**=**	**2 NAD(H) GENERATED / mol GLUCOSE**

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Quantitative Metabolism

In beer making, the Pyruvate produced is converted to Ethanol by the following reaction sequence:

Pyruvate + NAD(H) = Ethanol + CO_2

When coupled with the production step:

Glucose = 2 Pyruvate + 2ATP + 2 NAD(H)

2 Pyruvate + 2 NAD(H) = 2 Ethanol + 2 CO_2

Glucose = 2 Ethanol + 2 CO_2 + 2 ATP

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Quantitative Metabolism

This overall process is REDOX NEUTRAL

Ethanol is only one example of the production of end products which enable the generated NAD(H) to be used in end product formation.

The NAD^+ / NAD(H) pool is very small and must be tightly controlled to keep the NAD(H) generating processes and NAD(H) utilising processes in balance.

Similarly,

The ADP / ATP pool is very small and must be tightly controlled to keep the ATP generating processes and ATP utilising processes in balance.

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Quantitative Metabolism

FERMENTATION	PRODUCTS	MICROORGANISMS
Homolactic	Lactate	Streptococcus, Lactobacillus
Mixed Acid	Lactate, Acetate, Succinate, Formate, Ethanol, CO_2, H_2	Eschericia, Proteus, Shigella, Salmonella
Butanediol	As Mixed Acid Butanol, CO_2	Klebsiella, Serratia, Erwinia
Butanol	Acetate, Butyrate, Butanol, Acetone, Ethanol, i-Propanol	Clostridium
Butyric	Acetate, Butyrate, CO_2, H_2	Clostridium
Propionic	Acetate, Propionate, Succinate, CO_2	Propionibacterium, Veillonella
Ethanol	Ethanol, CO_2	Yeast

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Quantitative Metabolism

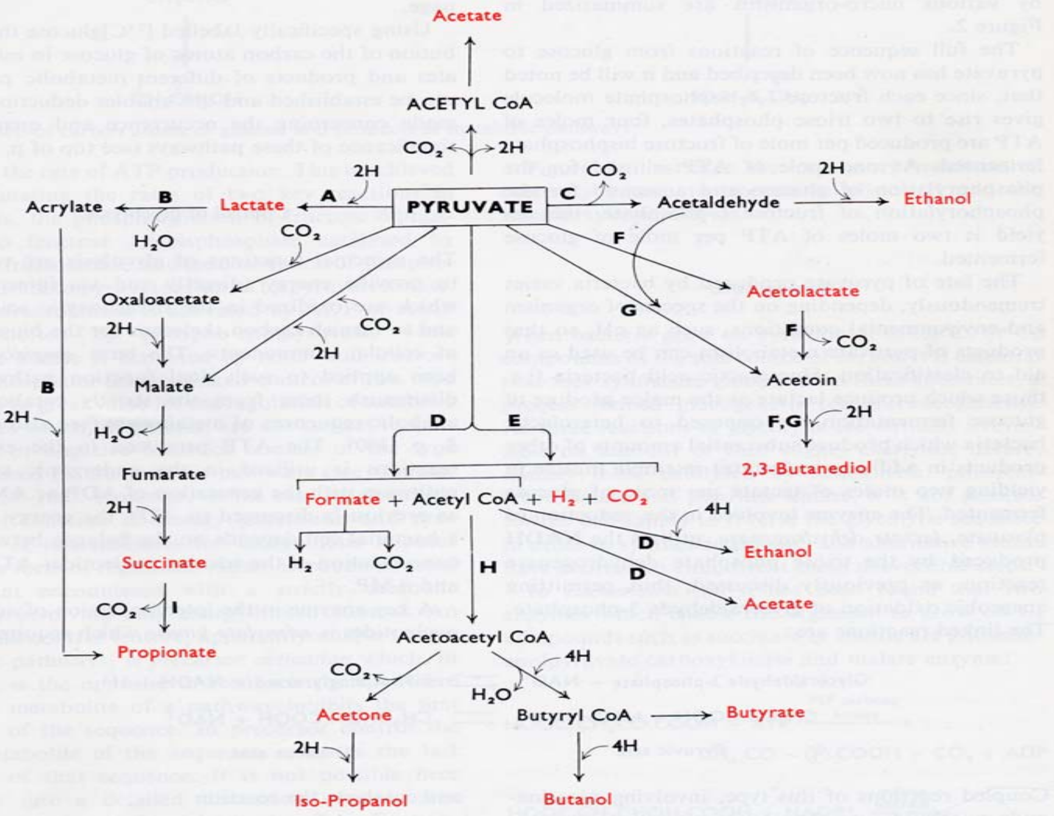

Figure 2. Bacterial fermentation products of pyruvate. Pyruvate formed by the catabolism of glucose is further metabolized by pathways which are characteristic of particular organisms and which serve as a biochemical aid to identification. End products of fermentations are indicated in red.

A Lactic acid bacteria (*Streptococcus*, *Lactobacillus*)
B *Clostridium propionicum*
C Yeast, Acetobacter, Zymomonas, *Sarcina ventriculi*, *Erwinia amylovora*.
D Enterobacteriaceae (coli-aerogenes)
E Clostridia
F Klebsiella
G Yeast
H Clostridia (butyric, butylic organisms)
I Propionic acid bacteria

End Product Formation

Quantitative Metabolism

As an alternate as being used for the generation of end products, Pyruvate may be further metabolised using other pathways. The most common pathway for this purpose is the Krebs or TCA Cycle.

To enter the TCA Cycle, the Pyruvate must be first transformed to acetyl Co A:

Pyruvate + CoA= Acetyl CoA + CO_2 + NAD(H)

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Quantitative Metabolism

Coenzyme A has the following structure:

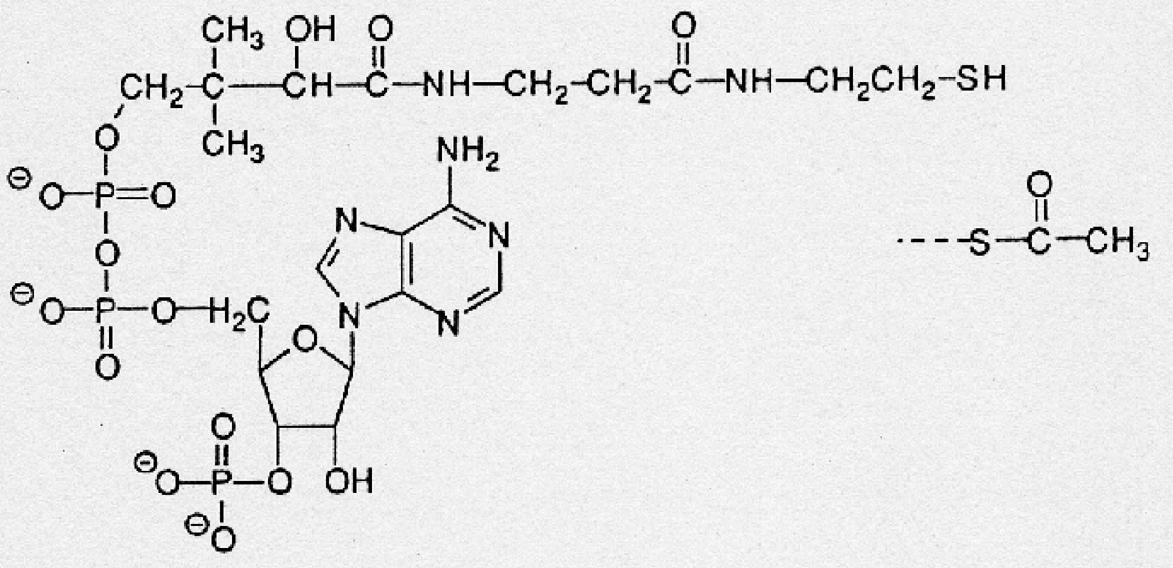
		Adenine	β Mercaptoethylamine
Phosphate	-	Ribose	Panthothenic Acid
		Phosphate -	Phosphate

The active part is the –SH group of the β Mercaptoethylamine molecule :

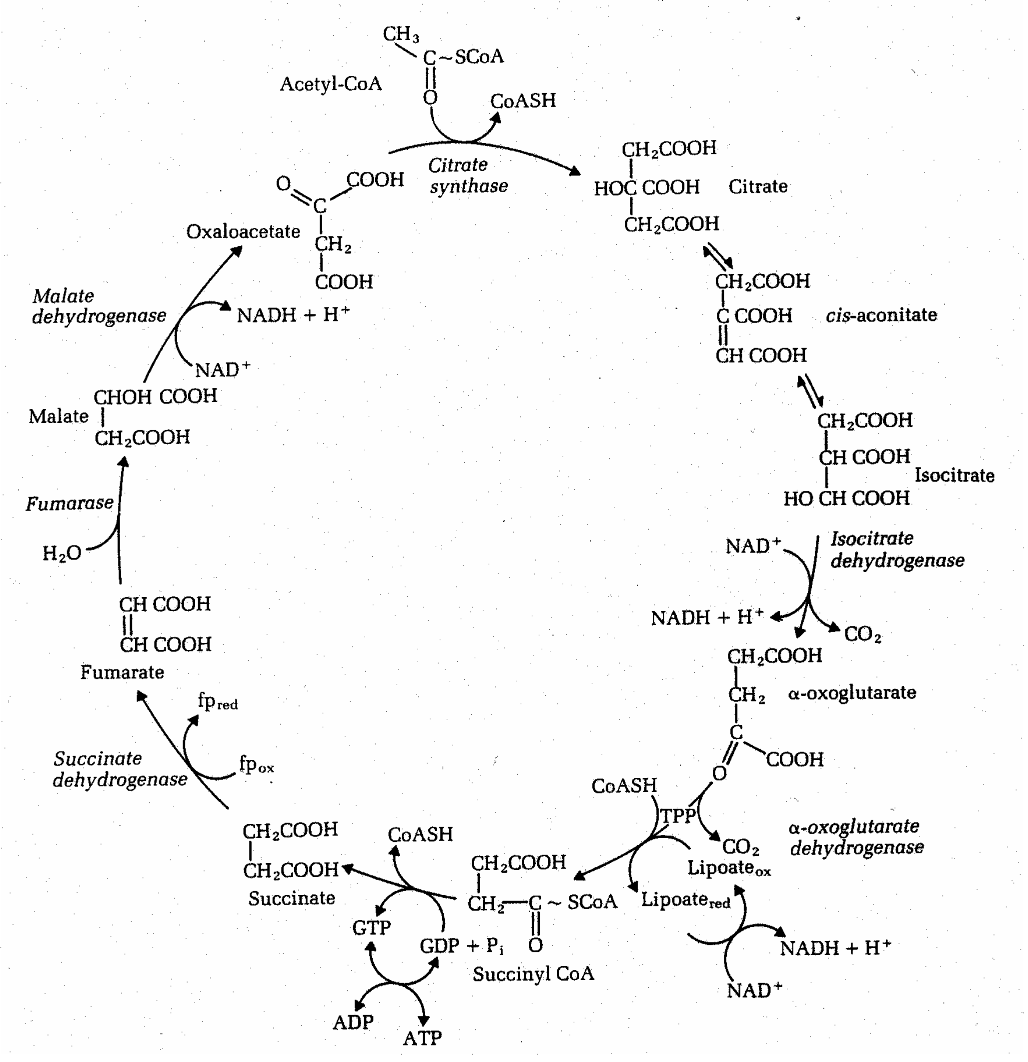
$$RCO - NHCH_2CH_2 - SH \quad = \; CoA - SH$$

Quantitative Metabolism

b. Coenzyme A and acetylcoenzyme A



Quantitative Metabolism



Quantitative Metabolism

In the TCA Cycle:

Acetyl CoA = 2 CO_2 + CoA + 1ATP +4NAD(H)

Carbon Source	**:**	**Acetyl**
Energy Source	**:**	**Acetyl**
End Product(s)	**:**	**2 CO_2**
Energy Formation (DIRECT)	**:**	**1 ATP generated / mol Acetyl**
Energy Formation (INDIRECT)	**:**	**4 NAD(H) generated / mol Acetyl (3NAD(H) and 1 FAD(H))**

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Quantitative Metabolism

How is INDIRECT energy converted to ATP ??

THIS IS A COMPLEX BIOLOGICAL PHENOMENA, INVOLVING THE TRANSFER OF PROTONS ACROSS A BIOLOGICAL MEMBRANE. PROTEIN COMPLEXES IN THESE MEMBRANES ARE CAPABLE OF *TURNING A HYDROGEN ION GRADIENT INTO ATP*

The mechanism may be represented as follows:

$$NAD(H) + H^+ = NAD + 2H^+ + 2\,e^-$$

$$(2\,H^+)_{\text{inner membrane}} = n\,(2\,H^+)_{\text{outer membrane}}$$

$$n\,(2\,H^+)_{\text{outer membrane}} = n\,(2\,H^+)_{\text{inner membrane}} + m\ ATP$$

The STOICHIOMETRY of this reaction (that is, the values for m ,n) are VARIABLE – hence the difficulties of predicting aerobic yields.

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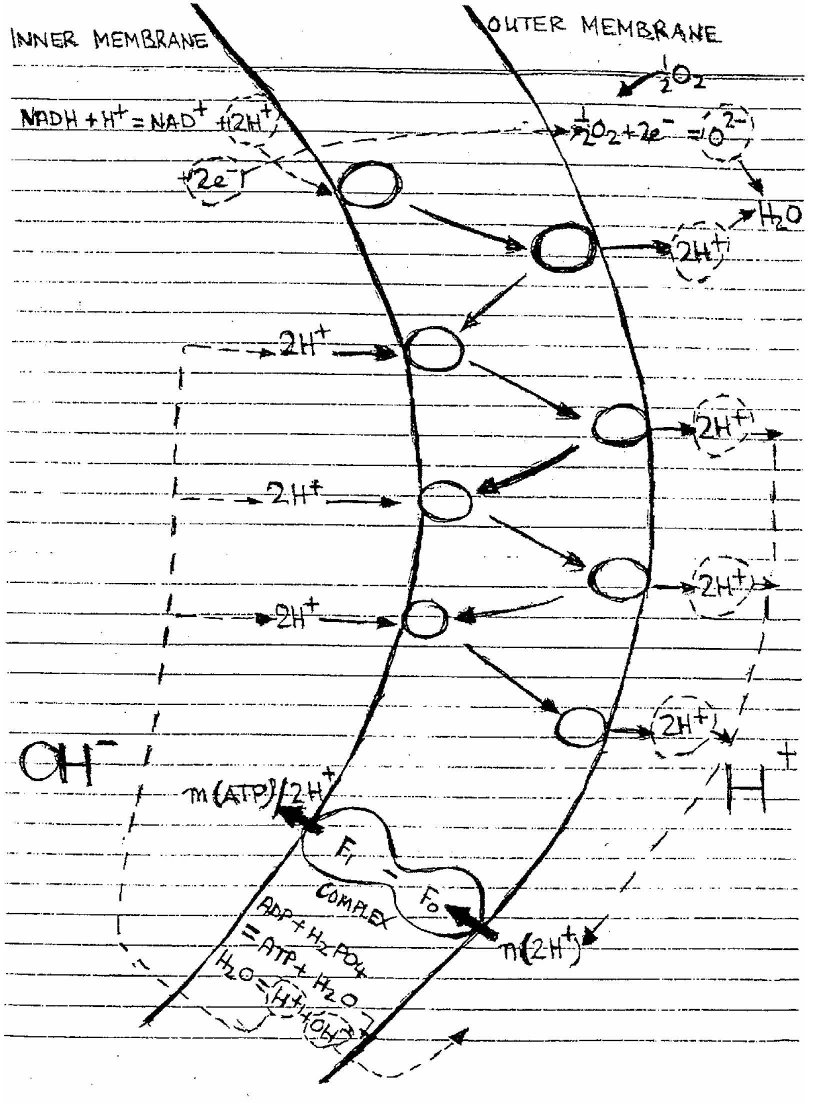
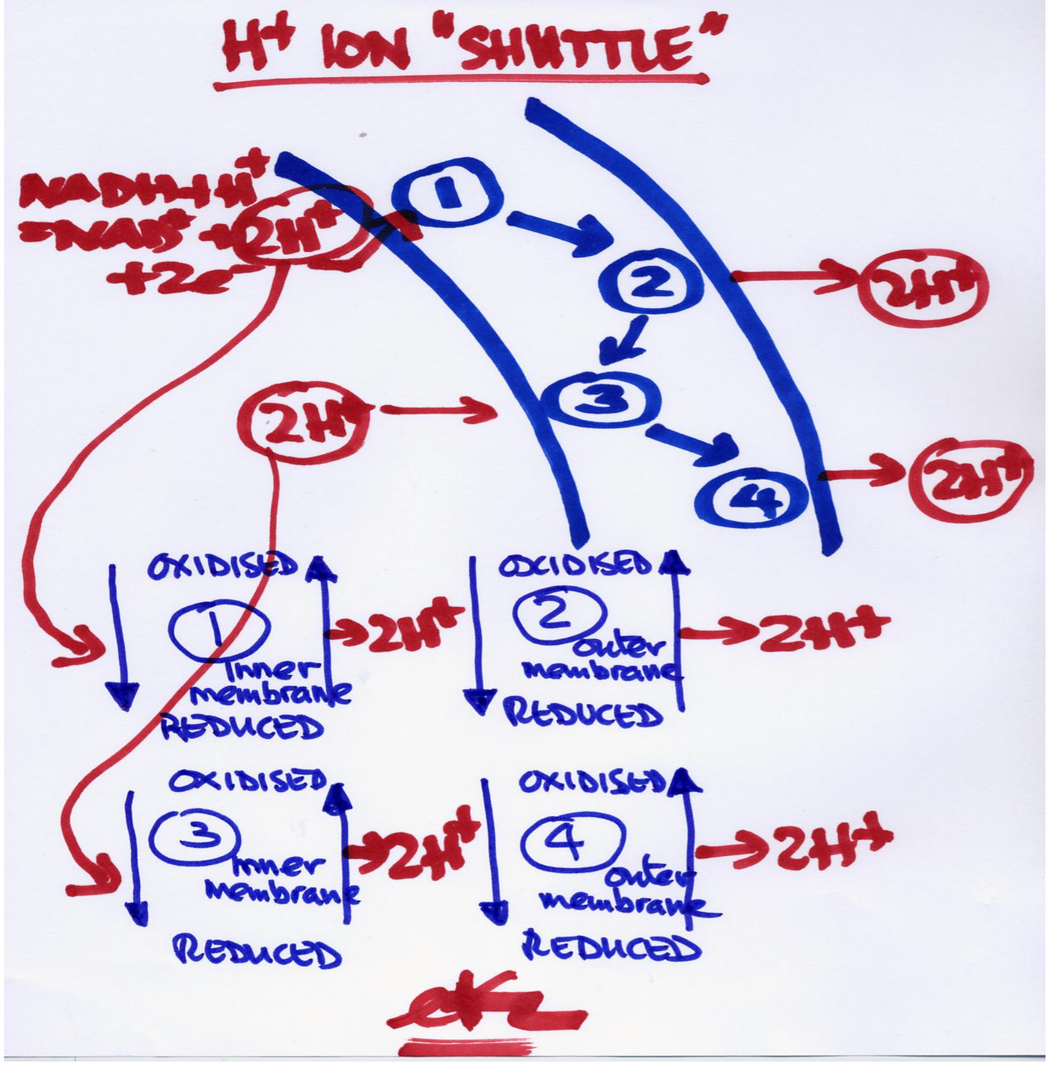
Quantitative Metabolism



Quantitative Metabolism



Quantitative Metabolism

The electrons generated by the reaction:

$NAD(H) + H^+ = NAD^+ + 2H^+ + 2e^-$

need to be utilised or “accepted” by a “terminal electron acceptor”

This is most commonly OXYGEN but a range of other “terminal electron acceptors” are possible

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Quantitative Metabolism

The mechanism for the use of OXYGEN is the following:

$$\tfrac{1}{2}\, O_2 + 2\, e^- = O^{2-}$$

$$O^{2-} + 2\, H^+ = H_2O$$

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Quantitative Metabolism

Other Electron Generating and Electron Accepting Processes

Sulfate Reducing Organisms:

Here SO_4^{2-} is used as follows:

$$SO_4^{2-} + 8H^+ + 8e^- = S^{2-} + 4H_2O$$

Nitrifying Microorganusms:

Ammonia → Nitrite

$$NH_4^+ + 2H_2O = NO^{2-} + 8H^+ + 6e^-$$

This process is undertaken by Nitrosomas

Nitrite → Nitrate

$$NO^{2-} + H_2O = NO^{3-} + 2H^+ + 2e^-$$

This process is undertaken by Nitrobacter

These hydrogen ions and electrons are normally taken up by OXYGEN.

Denitrifying Microorganisms:

Nitrate → Nitrogen

$$NO^{3-} + 6H^+ + 5e^- = N_2 + 3H_2O$$

Nitrite → Nitrogen

$$NO^{2-} + 4H^+ + 3e^- = N_2 + 3H_2O$$

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Nitrification and Denitrification

Nitrification is an aerobic process (requiring oxygen).
The overall reactions are the following:
Nitrification:

$NH_3 + 1.5O_2 = HNO_2 + H_20$
$HNO_2 + 0.5O_2 = HNO_3$

What actually happens in terms of H+ and e- is the following:
Nitrification:
$NH_3 + 2H_2O = HNO_2 + 6H^+ + 6e^-$
$NO_2 + H_2O = HNO_3 + 2H^+ + 2e^-$

Denitrification:
$2HNO_3 + 10H^+ + 10e^- = N_2 + 6H_20$

Hence Nitrification **produces** H^+ and e^- and Dentrification **requires** H^+ and e^-.

As usual, these H^+ and e^- come from the reaction:

$NAD(H) + H^+ = NAD^+ + 2H^+ + 2e^-$

The balanced reactions for nitrification and denitrification (in terms of NAD(H) and NAD+) then become:

Nitrification:

$NH_3 + 3NAD^+ + 2H_2O = HNO_2 + 3NAD(H) + 3H^+$

$HNO_2 + NAD^+ + H_2O = HNO_3 + NAD(H) + H^+$

Denitrification:

$2HNO_3 + 5NAD(H) + 5H^+ = N_2 + 6H_2O + 5NAD^+$

In nitrification, oxygen is used to regenerate the NAD(H) formed:

$NAD(H) + H^+ = NAD^+ + 2H^+ + 2e^-$
$0.5O_2 + 2e^- = O_2^-$
$O_2^- + 2H^+ = H_2O$

$NAD(H) + 0.5\ O_2 + H^+ = NAD^+ + H_2O$

In nirtrification, there is a nett use of NAD(H) from the energy generating pathways (using CO2 as a carbon source) and this is provided by the nitrification reaction.

In denitrification, a carbon and energy source provides the NAD(H) required to drive the denitrification reaction.

Quantitative Metabolism

It should be clear that for Denitriification to occur there needs to be a source of hydrogen ions and electrons.

These may come from NAD(H) (which may be produced by the energy producing pathways).

In anaerobic systems, where oxygen is not present:

Either

The electrons must be taken up by some other EXTERNAL electron acceptor (e.g SO_4^-)

OR

Any NAD(H) produced must be by catabolism must be accepted by END PRODUCTS (e.g. Ethanol formation)

OR

The carbon and energy source is split between a high oxidation product and a low oxidation state product.

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Quantitative Metabolism

The breakdown of acetate to CO_2 and CH_4 by METHANOGENIC BACTERIA is a very good example of this:

Substrates | **Products**

ACETATE ($C_2H_4O_2$) → **CO_2 (HIGH OXIDATION STATE)**

($C_2H_4O_2$)

→ **CH_4 (LOW OXIDATION STATE)**

The oxidation state of the products is equal to the oxidation state of the substrates.