UNIT-IV (Part-I)

Catabolism of glucose: Breakdown of glucose to produce energy.

The respiration (aerobic) of glucose occurs in three principal stages:

1. Glycolysis 2. the Krebs cycle 3. the electron transport chain

GLYCOLYSIS: is the oxidation of glucose to pyruvic acid with the production of ATP and energy containing NADH, in a ten-step process/pathway. Also called Embden-Meyerhof pathway.

- Glycolysis does not require oxygen; it can occur whether oxygen is present or not.
- Glycolysis consists of 2 basic stages:

(i) Preparatory stage: Two molecules of ATP are used as 6-carbon glucose is phosphorylated, restructured & split into two 3-carbon compounds, Glyceraldehyde-3-phosphate(GP) and dihydroxyacetone phosphate (DHAP). DHAP is readily converted to GP.

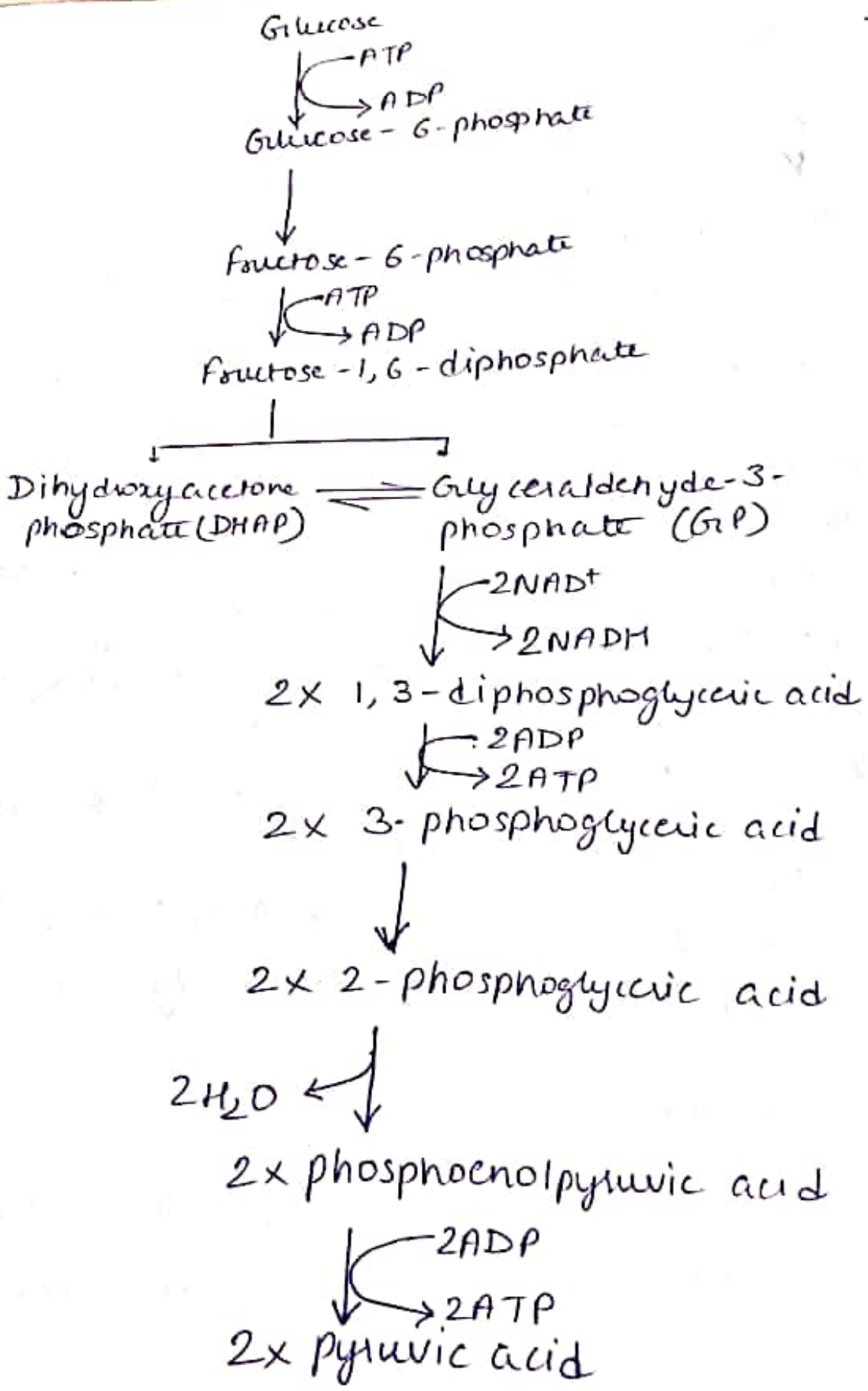
(ii) Energy conserving stage: 2 molecules of GP are oxidized in several steps to two molecules of pyruvic acid. Two molecules of NAD^+ are reduced to NADH & four ATP are formed by substrate level phosphorylation.

Net gain of ATP:

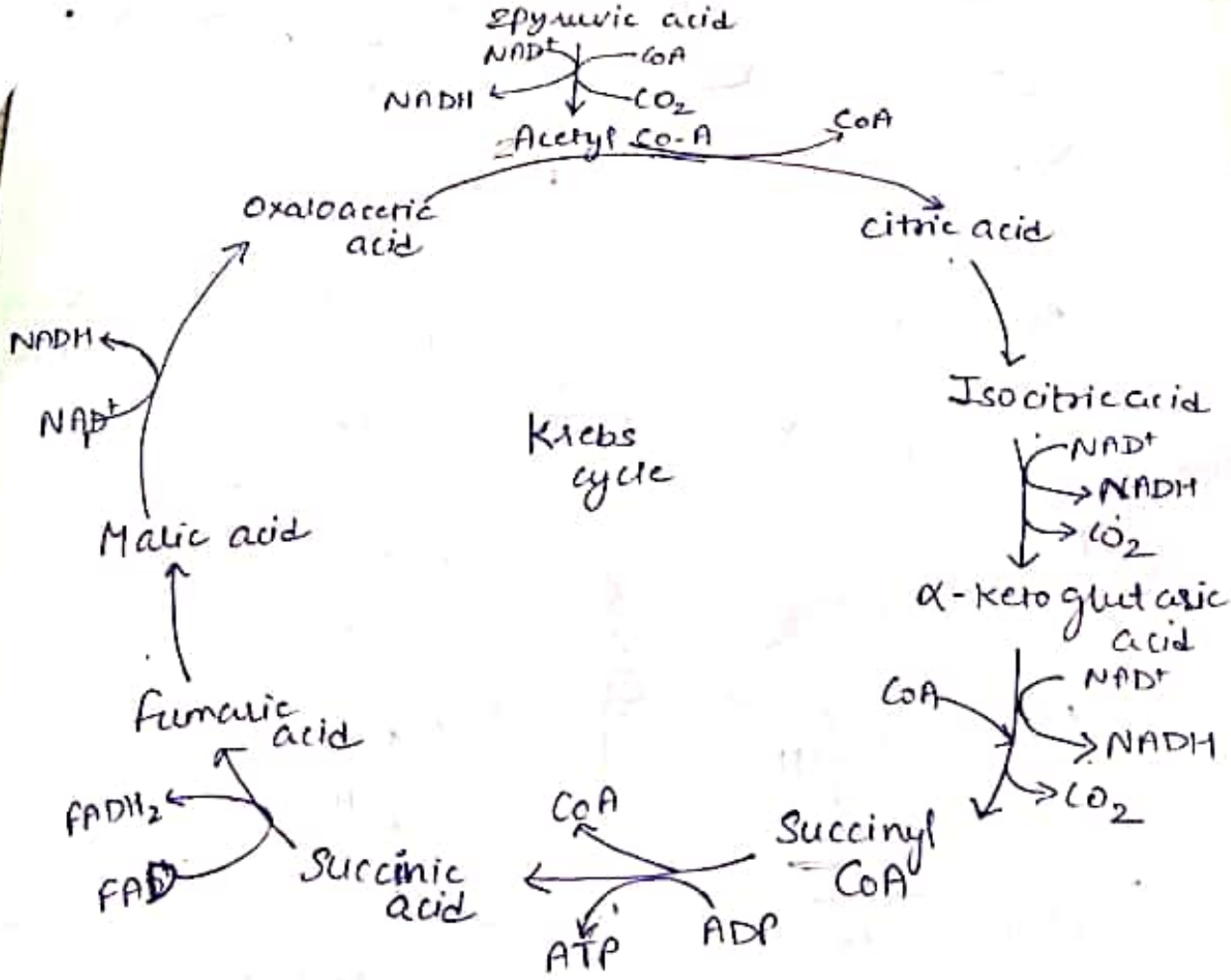
(i) by substrate level phosphorylation = 4 − 2 = 2 ATP

(ii) by oxidative phosphorylation = 2NADH = 2 × 3 = 6 ATP

8 ATP



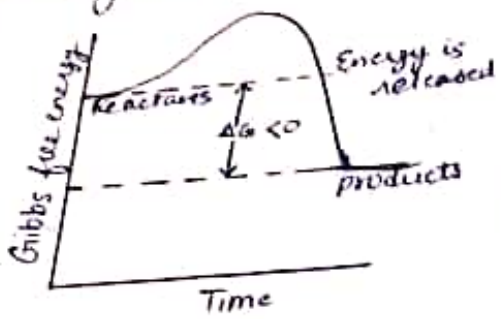
KREBS cycle : also called tricarboxylic acid (TCA) e or citric acid cycle is a series of reactions in ich the large amount of potential chemical energy tored in acetyl CoA is released step by step.



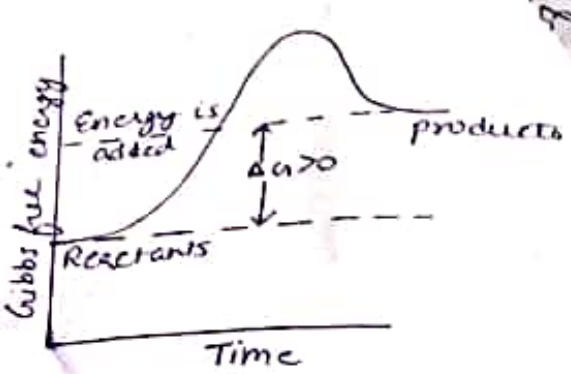
- For every 2 molecules of acetyl Co-A that enter the cycle: - 4 molecules of CO_2 are liberated

- 2 ATP	produced	= 2 ATP
- 6 NADH		= 18 ATP
- 2 $FADH_2$		= 4 ATP
		Net gain = (24 ATP)

4 Concept of Standard free energy & spontaneity of Biological reactions

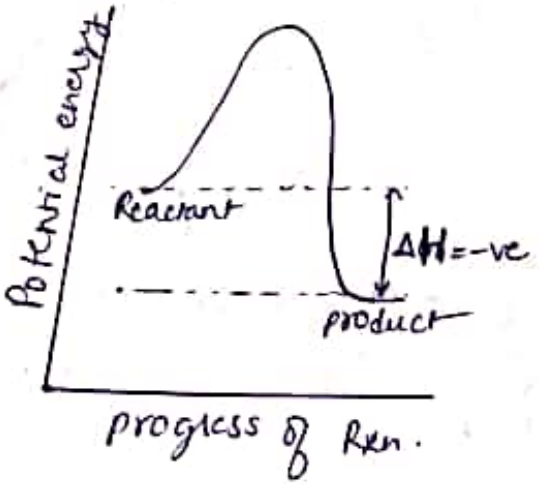


Exergonic reaction ΔG<0
Reaction is spontaneous



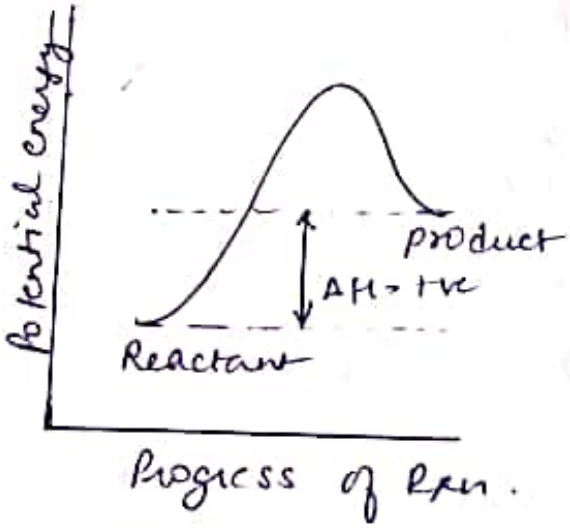
Endergonic reaction ΔG>0
Reaction is not spontaneous

Concept of Exothermic & endothermic reaction.



Exothermic reaction

→ Heat energy is released



Endothermic reaction.

→ Heat energy is consumed.

Synthesis of glucose : PHOTOSYNTHESIS :
synthesis of carbohydrates by green plants in the presence of light & chlorophyll by utilising CO_2 & H_2O.
It occurs in 2 phases : ① Light Reaction
② Dark Reaction.

Light Reaction : Photophosphorylation :
In Light reaction occurs only in photosynthetic cells
In light reaction, light & energy is absorbed by chlorophyll molecules in the photosynthetic cells, exciting some of the molecules' electron. The excited electrons jump from a chlorophyll (Photosystem, PS) to a series of carrier molecules. As electrons are passed along series of carriers, protons are pumped across the membrane & ADP is converted to ATP by chemiosmosis. It is of 2 types:

Cyclic photophosphorylation: In this, cyclic photophosphoelectron eventually returns to the chlorophyll (PS)

Non-cyclic photophosphorylation: In non-cyclic photophosphorylation, electrons released from chlorophyll (PS) do not return to chlorophyll but become incorporated into NADPH.

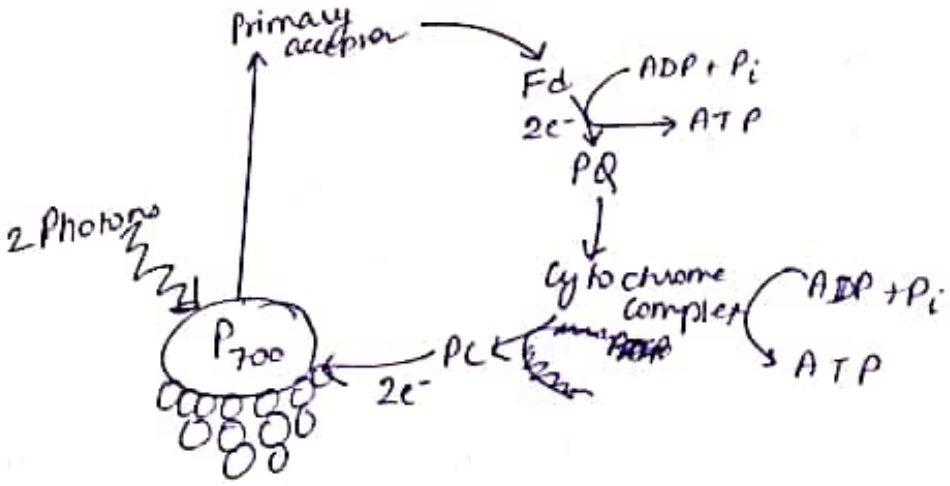
The oxidised P_{680} (chlorophyll) regains its electrons by photolysis of water into $2H^+$, $2e^-$ & oxygen.

As a result of light rxn, following end products are formed :

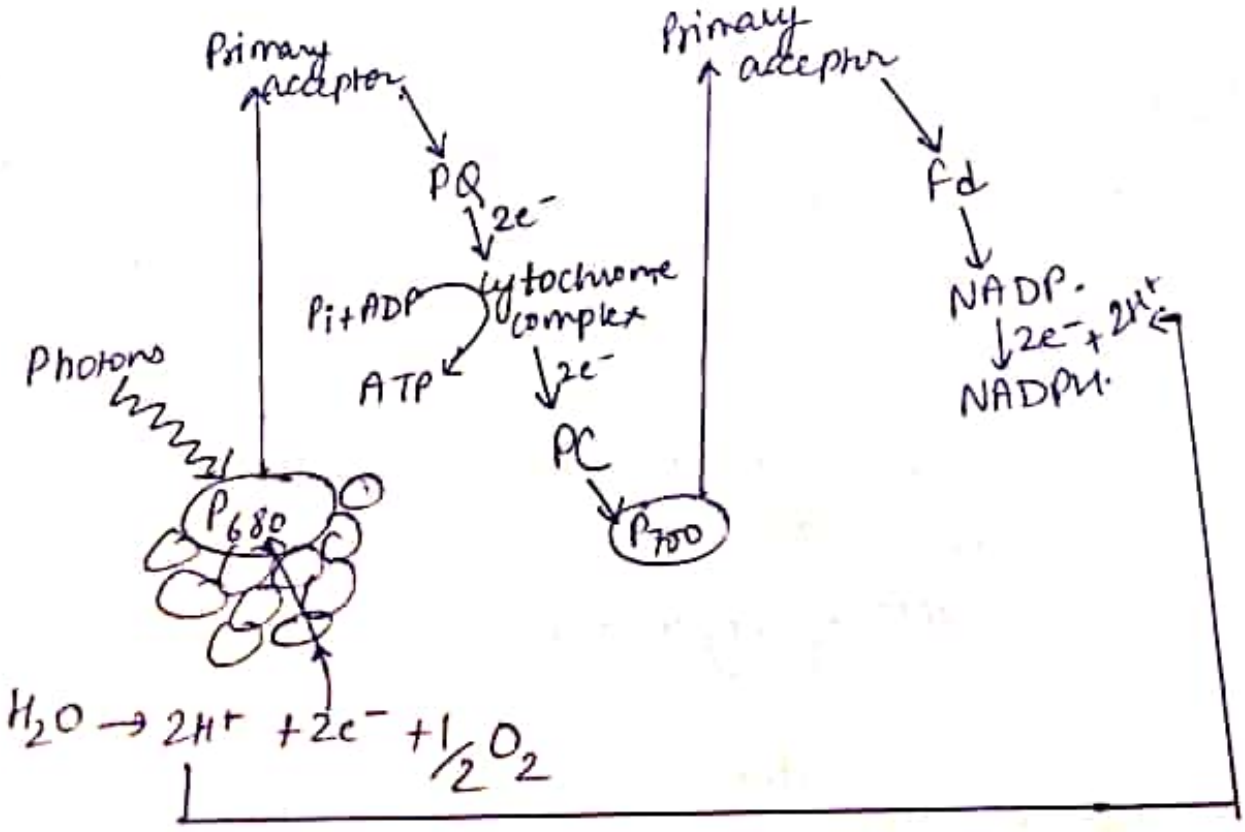
① Oxygen → released into atmosphere
② NADPH } utilised for reduction of CO_2 to
③ ATP } carbohydrate in dark reaction.

Fd = Ferredo... light-independers
PS = Plasto quin... Calvin-Benson c...
PC = Plasto cyan... so named be...
light.
reducing
are
a co...

6 Cyclic photophosphorylation.



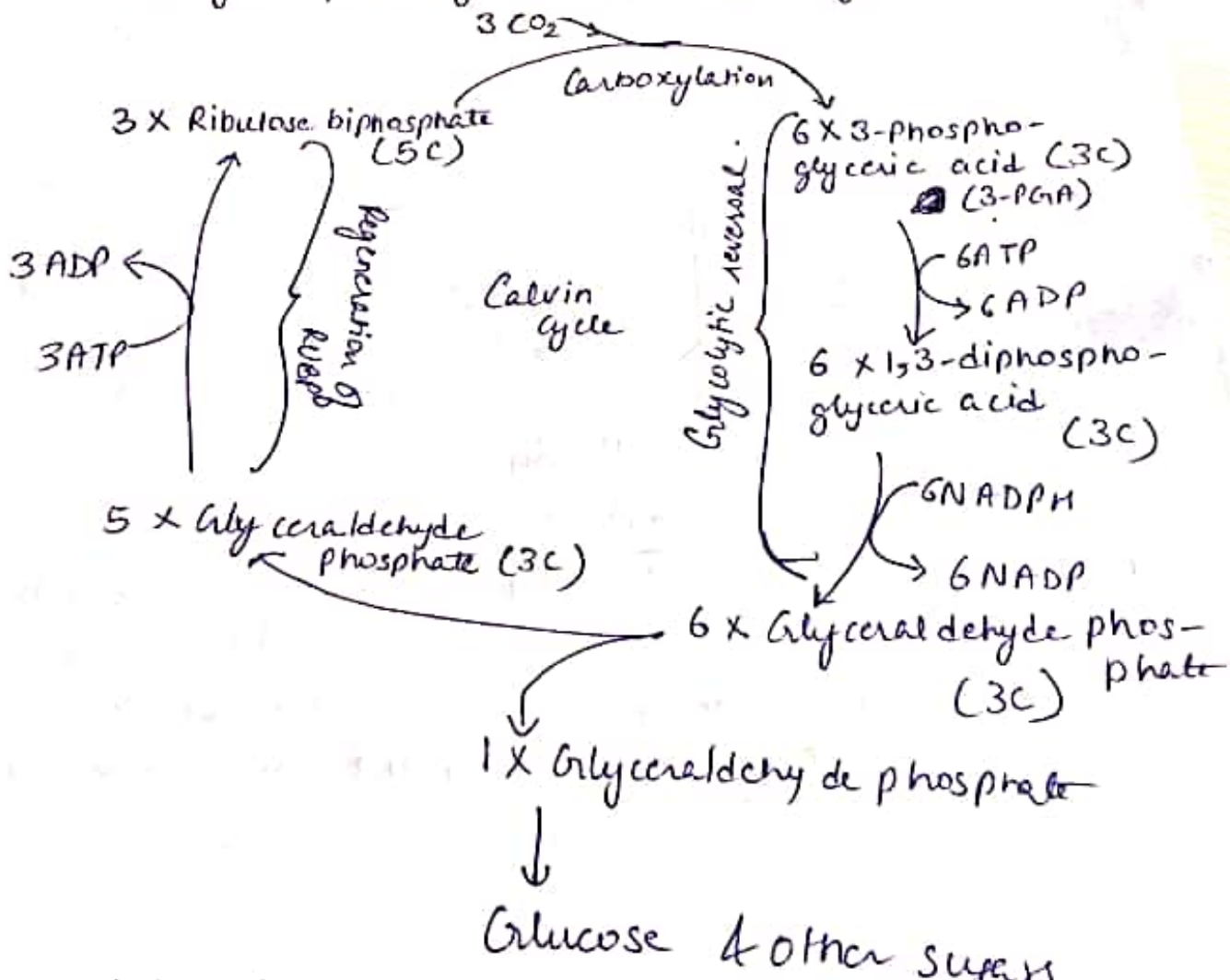
Non-cyclic photophosphorylation



Fd = Ferredoxin
PQ = Plastoquinone
PC = Plastocyanin

Light-independent Reaction / Dark Reaction / Calvin-Benson cycle / C_3 cycle:

- So named because it is not dependent upon light.
- Reducing capacity of NADPH & energy in form of ATP (produced in light rxn) are used to convert CO_2 into carbohydrates by a complex cyclic pathway called Calvin cycle.



It consists of three parts:

1. Carboxylation
2. Glycolytic reversal.
3. Regeneration of RUBP

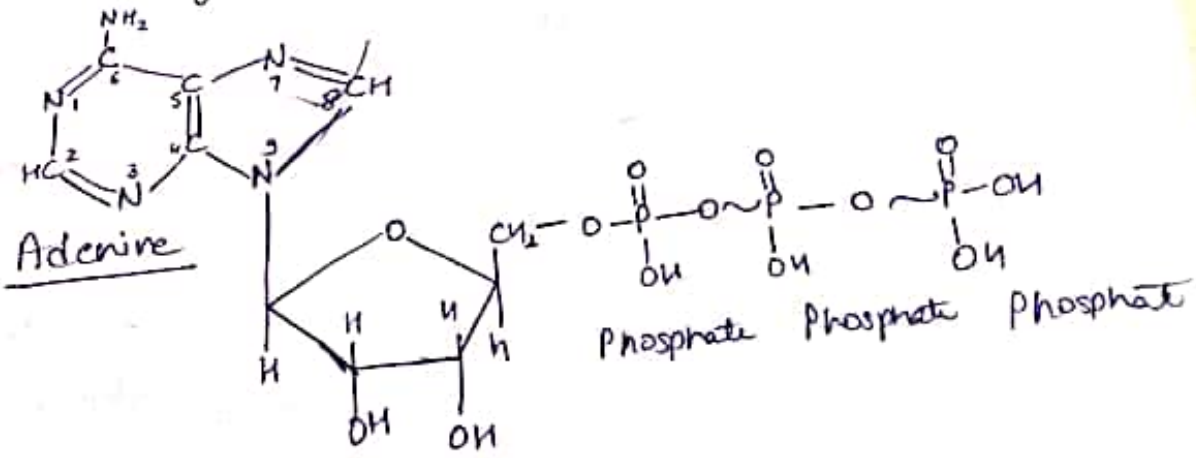
3 ATP as energy currency of the cell

Adenosine triphosphate (ATP) is known as energy currency of the cell because it can trap, store and release small packets of energy

Chemically ATP is made up of adenine, linked to ribose, which is linked to a string of three phosphate radicals. Out of three phosphate radicals, the last two are attached by means of 'energy rich bonds' ∵ of 2 reasons.

(i) mutual repulsion of positive charges gained by adjacent phosphorus due to dipole formation with oxygen.

(ii) loss of resonance by phosphate radicals in the combined state.



The bond b/w the second & third phosphates can be easily broken to liberate energy. $ATP + H_2O \xrightleftharpoons{ATPase} ADP + P_i + Energy$

The breakage of last energy rich bond releases an energy of 8.9 Kcal/mol, while the second energy rich bond has energy equivalent of 6.5 kcal/mol.

Microbiology: Concept of species & strain:

According to Bergey's manual of Systematic Bacteriology:
A strain is made of descendants of a single isolation in pure culture and usually is made of succession of cultures ultimately derived from initial single colony.
'A single isolation in pure culture' indicates that the strain has been isolated from a particular site at a particular time.
A microbial species include group of strains from a variety of sources which have in common, a set of correlating stable properties that separates the group from other groups of strains.
The population of strain mentioned includes
- freshly isolated strain
- stock strains maintained in vitro
- & their variants

} with common set of properties → SPECIES.

Sterilization: Any process that kills or removes all forms of microorganisms such as bacteria, virus, fungi & their spores.

① Moist heat Sterilization:

Sterilization: Steam under high pressure is a reliable method for sterilization and can be achieved in an autoclave. Autoclaving is used to sterilize culture media, instruments, dressings, syringes etc.

The sterilizing temperature & pressure in an autoclave is 121°C & 15psi (pounds per square inch). Under these conditions, steam at high pressure will kill all microorganisms & their endospores in about 15 minutes.

10 (a) Dry heat sterilization:

(i) by direct flaming: for eg: to sterilize the inocula loop. wire is heated to red glow.

(ii) Hot-air sterilization: Items to be sterilized are placed in an oven. Temperature of about 170°C is maintained for around 2 hours to ensure sterilization.

Media composition:

A nutrient material prepared for the growth of microorganisms in a lab is called culture medium.

The microbes that grow & multiply in/on a culture medium are referred to as culture.

A medium must provide an energy source as well as source of carbon, nitrogen, sulfur, phosphorus and organic growth factors the organism is unable to synthesize. In addition, for preparing solid media, a solidifying agent such as agar is added to the medium.

A complex media is made up of nutrients such as extracts from yeast, meat or digest of proteins from these & other sources.

- If complex medium is is Liquid form it is called nutrient broth
- When agar is added, it is called nutrient agar.

Following is the composition of nutrient broth & nutrient agar.

Nutrient broth

Beef extract - 3g
Peptone - 5g
Water - 1000ml

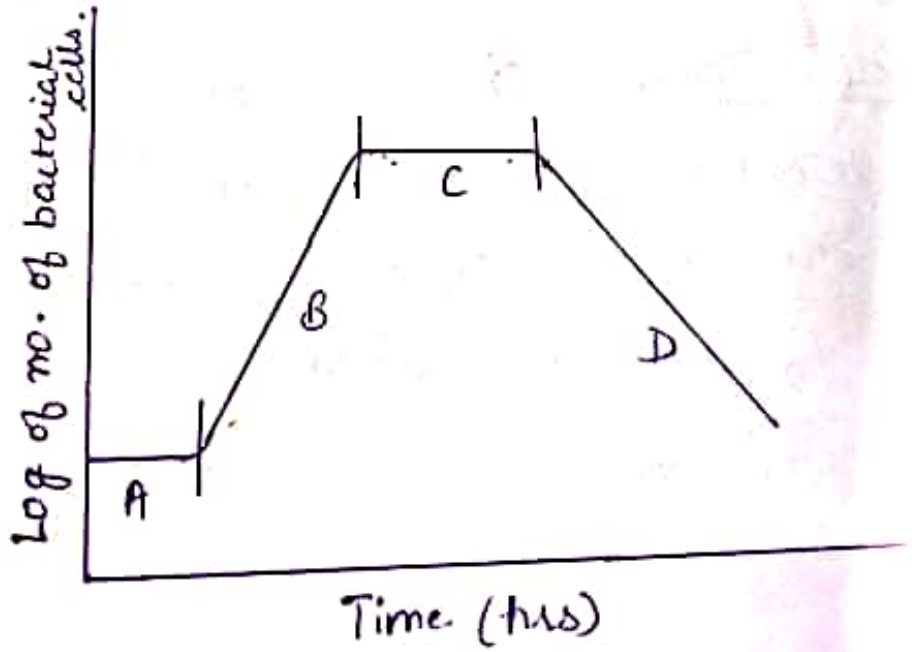
Nutrient agar

Beef extract - 3g
Peptone - 5g
Agar - 15g
Water - 1000ml

Characteristics of several complex materials used as ingredients of media:

Raw Material	Characteristics	Nutritional Value
(1) Yeast extract	An aqueous extract of yeast cells that has been concentrated to a paste	Contains the water soluble substances of animal tissue, which include carbohydrates, organic nitrogen compounds, water soluble vitamins and salts
(2) Peptone	The product resulting from the digestion of proteinaceous materials, e.g., meat, casein and gelatin; digestion of the protein material is accomplished with acids or enzymes; many different peptones (depending upon the protein used and the method of digestion) are available for use in bacteriological media; peptones differ in their ability to support growth of bacteria	Principal source of organic nitrogen, may also contain some vitamins and sometimes carbohydrates, depending upon the kind of proteinaceous material digested
(3) Agar	A complex carbohydrate obtained from certain marine algae; processed to remove extraneous substances	Used as a solidification agent for media; agar, dissolved in aqueous solutions, gels when the temperature is reduced below 45°C; agar is not considered a source of nutrients to the bacteria
(4) Yeast extract	An aqueous extract of yeast cells, commercially available as a powder	A very rich source of most B vitamins; also contains organic nitrogen and carbon compounds

GROWTH KINETICS: Typical bacterial growth consists of 4 phases:



12

Lag Phase (A) : The addition of inoculum to a new medium is not followed immediately by a doubling of the population. Instead, the population remains temporarily unchanged. During this stage, the individual cells increase in size beyond their normal dimensions. Physiologically, they are active & synthesize new protoplasm.

Logarithmic phase / Exponential phase (B) :

During this period the cells divide steadily at a constant rate and the log of the number of cells plotted against time results in a straight line.

Stationary phase (C) : The log phase begins to taper off due to exhaustion of some nutrients & sometimes due to production of toxic products during growth. The population remains constant for a time because reproduction rate is balanced by equivalent death rate.

Death / Decline phase (D) : Following the stationary phase, the bacteria may die faster than new cells are produced due to depletion of nutrients & accumulation of inhibitory products, such as acids. During this phase, the no. of cells decreases exponentially.