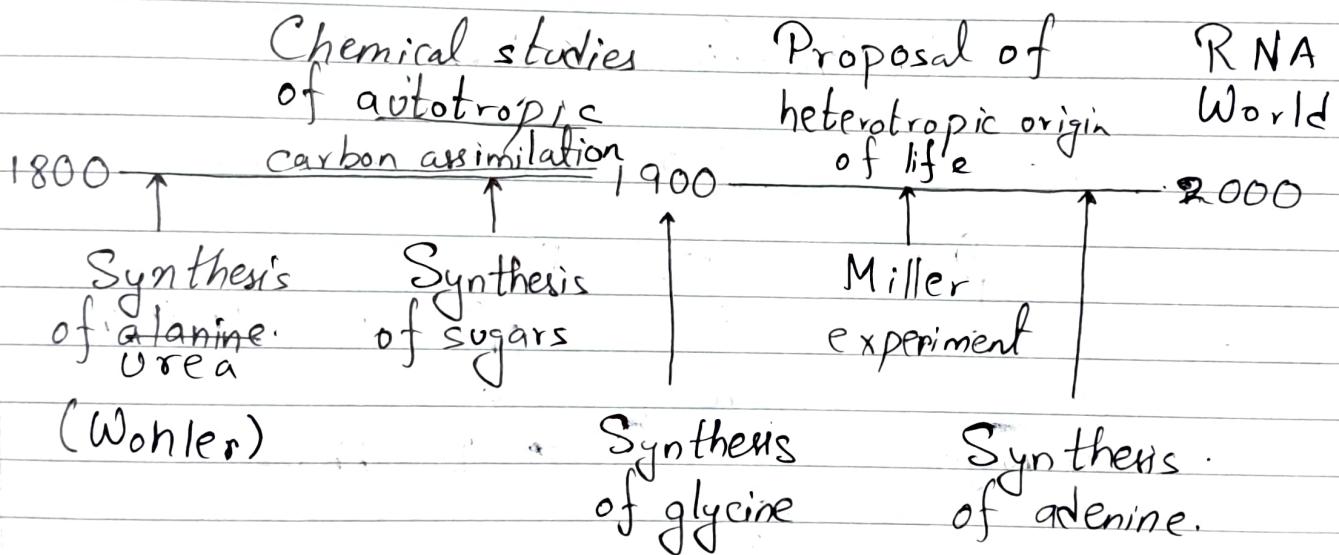


Biochemical Engg.

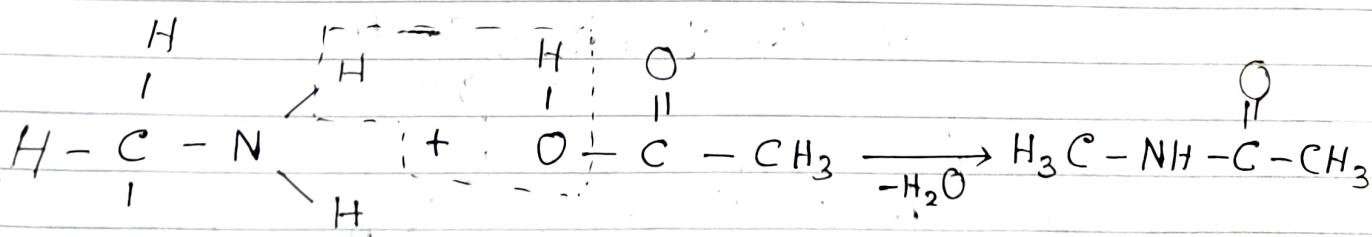
↳ Dealing with systems that have very short time span. (narrow range of pH , temp., heat, etc.)

Abiotic → Something that does not come from biology

Origin of biomolecules.



70% of biomass is proteins.

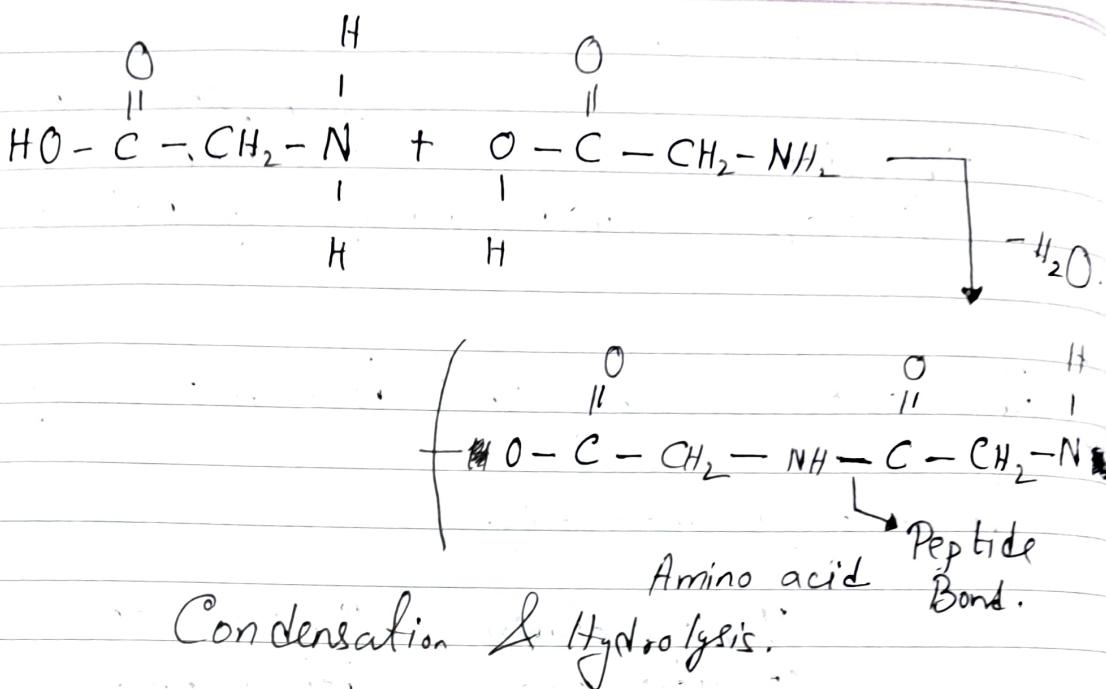


Methyl-amine

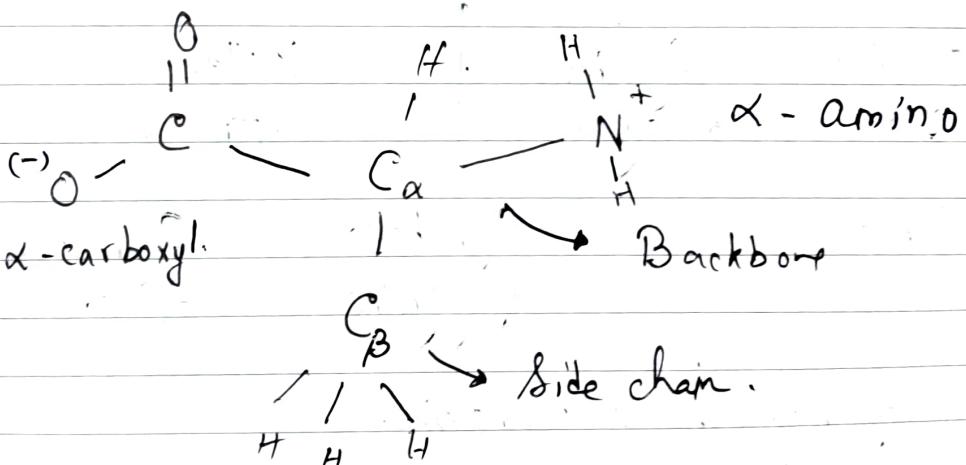
Acetic acid

Acetamide.

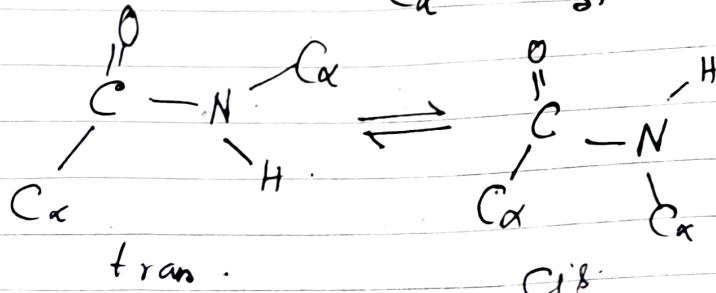
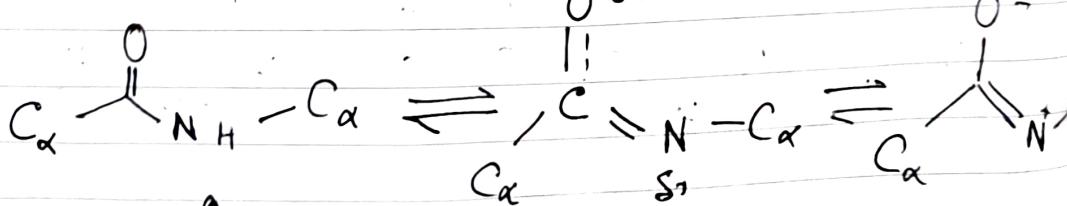
Condensation rxn.



There are 20 natural amino acids.



Peptide Bonds. (-C-N-)

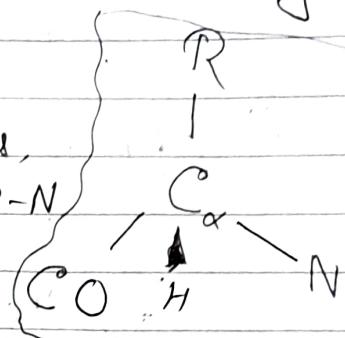
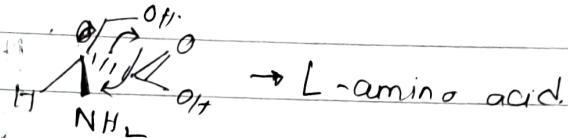
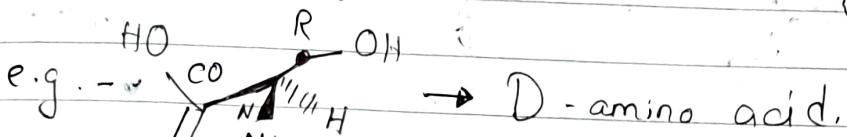


$t_{1/2}$ of hydrolysis of peptide bond ~ 350 yrs.

CORN Rule \rightarrow With H pointing at us,

if we can spell CO-R-N
in a clockwise fashion then it is

L-amino acid.



Natural amino acids.

Nonpolar side chains; hydrophobic.

Glycine (Gly or G)	Alanine (Ala or A)	Valine (Val or V)	Leucine (Leu or L)	Isoleucine (Ile or I)
-----------------------	-----------------------	----------------------	-----------------------	--------------------------

Methionine
(Met or M)

Phenylalanine
(Phe or F)

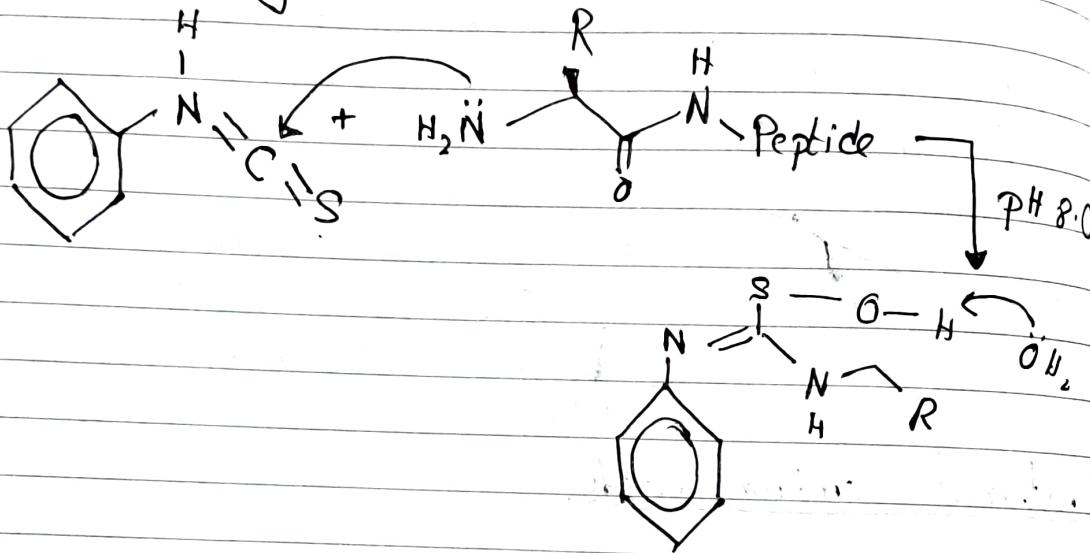
Tryptophan
(Trp or W)

Proline
(Pro or P)

Protein primary structure: sequencing.

Sequence of amino acids from N-terminal to C-

① Edman degradation.



Break only the first amino acid from N-terminal

→ tedious as there will be many monomers

② Mass Spectrometry.

→ Sep. on the basis of charge by mass

Fast A format.

First line:- Comment

Second line :- Amino acid residue identification in eight format.

80 characters per line.

Secondary Structure: Steric limitations. (Helices, Loops, Sheets)

$O = C - N - H$ lie on the same plane.
 → st. conf. of amino acid residues which are close neighbors.
 The $C\alpha$ group lie on diff. planes, having an angle of ϕ & ψ .

Helices are formed due to the O-H bond & rotation of $C\alpha$ plane.



Clockwise → Left

Anti-clockwise → Right

Most of the proteins are right handed α helix.

There are very few D-amino acids compound.

L-amino acids form right handed α helix.

i^{th} residue will make a hydrogen bond with $(i+4)^{th} C\alpha$.

3.6 res / $\frac{1}{4}$ turns

Pitch = 5.4 Å

B-sheets → Parallel sheet
 → Anti-parallel sheet

X-ray
crys. fall., etc.

Loops. :- No characteristic

Tertiary structure. (Polypeptide chain).

Two major structures:-

- (I) Fibrous.
- (II) Globular.

} → H-bonds
 } → Disulfide bonds.
 } → Van der Waals bond.

Quaternary structure. (Assembled subunits)

→ more than 1 polypeptide chain

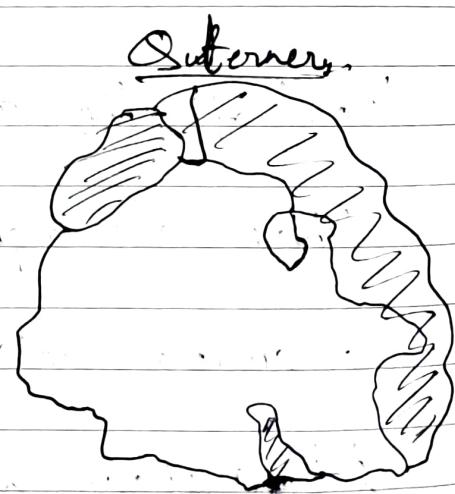
Primary Secondary Tertiary



α -helix

amino
acids.

Poly peptide
chain



Assembled
subunits

Determining crystal structure.

X-ray diffraction.

Protein folding - Anfinsen's experiment.

Ribonuclease $\xrightarrow{8\text{M urea}}$ Denatured reduced ribonuclease
 & β -mercaptoethanol
 breaks the H-bond $\xrightarrow{\text{breaks S-S bridges}}$
 \rightarrow complete 3-d structure.

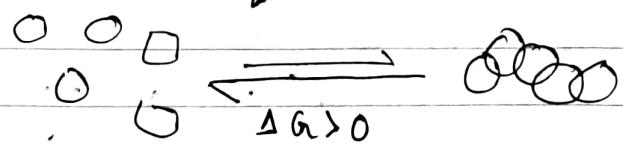
All proteins are polypeptides but all polypeptides are not proteins.

After removing the urea & β -mercaptoethanol, the protein folds again.

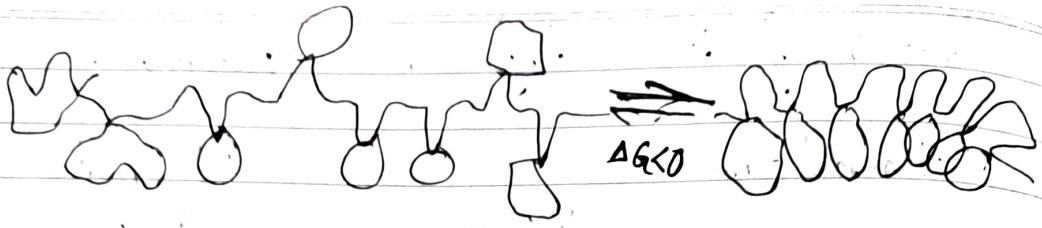
Corresponding to ~~there~~ a chain there is a unique 3-D structure.

Thermodynamic feasibility -

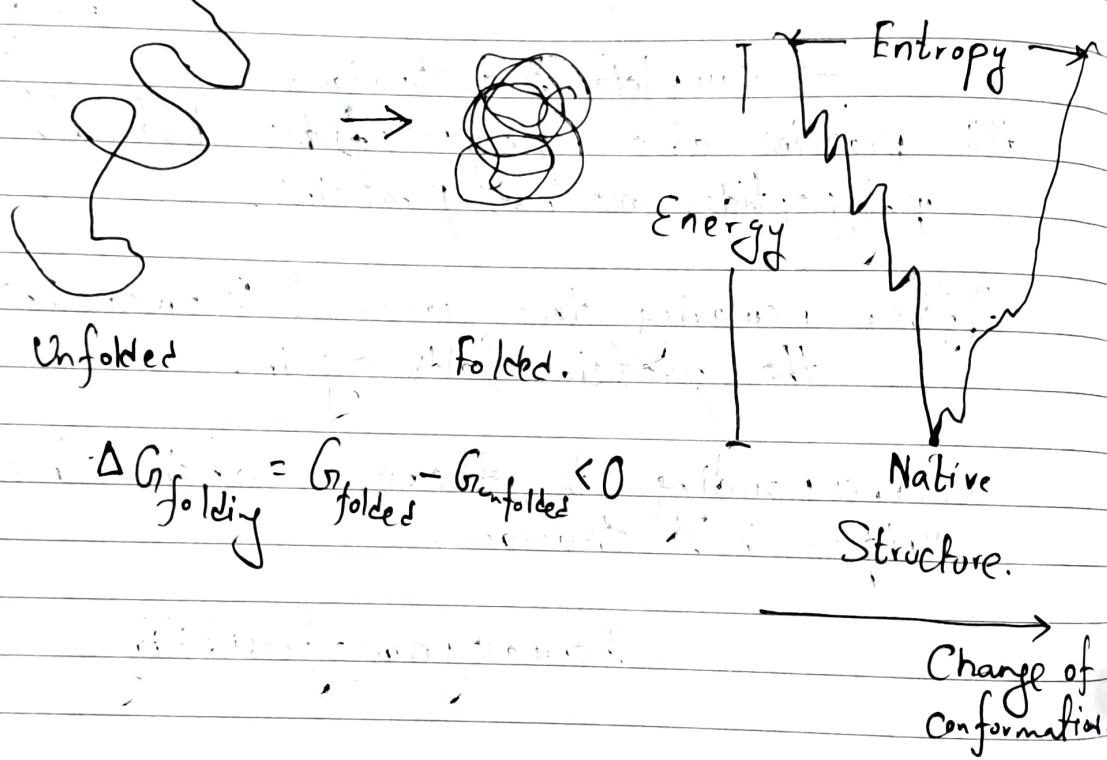
Thermodynamic potentials $\left\{ \begin{array}{l} \Delta E \rightarrow S, V \\ \Delta H \rightarrow S, P \\ \Delta A \rightarrow T, V \\ \Delta G \rightarrow T, P \end{array} \right.$ For the process to be spontaneous $\Delta G = \Delta H - T\Delta S < 0$.
 $\boxed{\begin{array}{l} \Delta S < 0 \\ \Delta H < 0 \end{array}}$



We need for $\Delta G < 0$, $\Delta H < 0$ & $\Delta S > 0$.



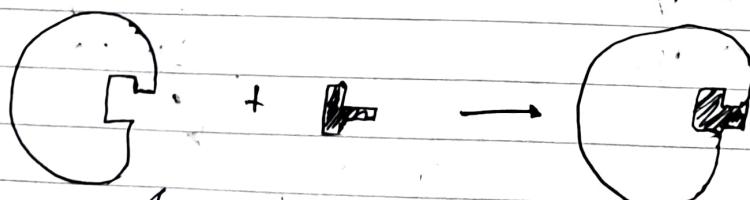
Funnel shaped energy landscape



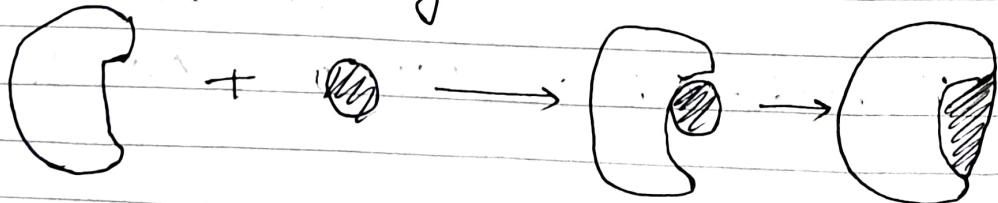
$$\Delta G_{folding} = G_{folded} - G_{unfolded} < 0$$

Lock & Key Mechanism

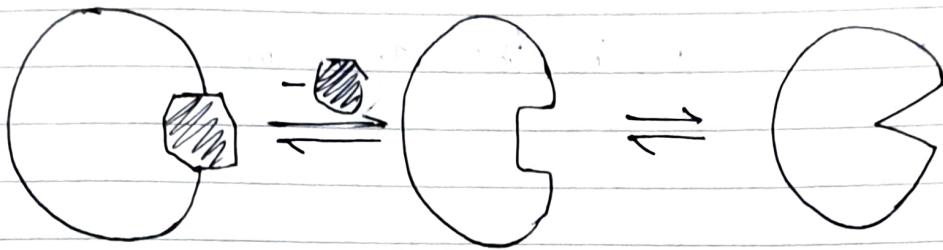
for Protein-substrate interactions



Induced key mechanism.



Conformational selection mechanism



In presence of substrate the protein shows only one of its many conformational structures.

e.g., Haemoglobin

"Promiscuity" of enzymes means they interact with more than 1 protein.



→ catalyses a reaction

Other mechanisms → geometric match
→ electrostatic match

Proteome → collection of all proteins in an organism.
↓
Protein.

Proteomics → study of proteome.

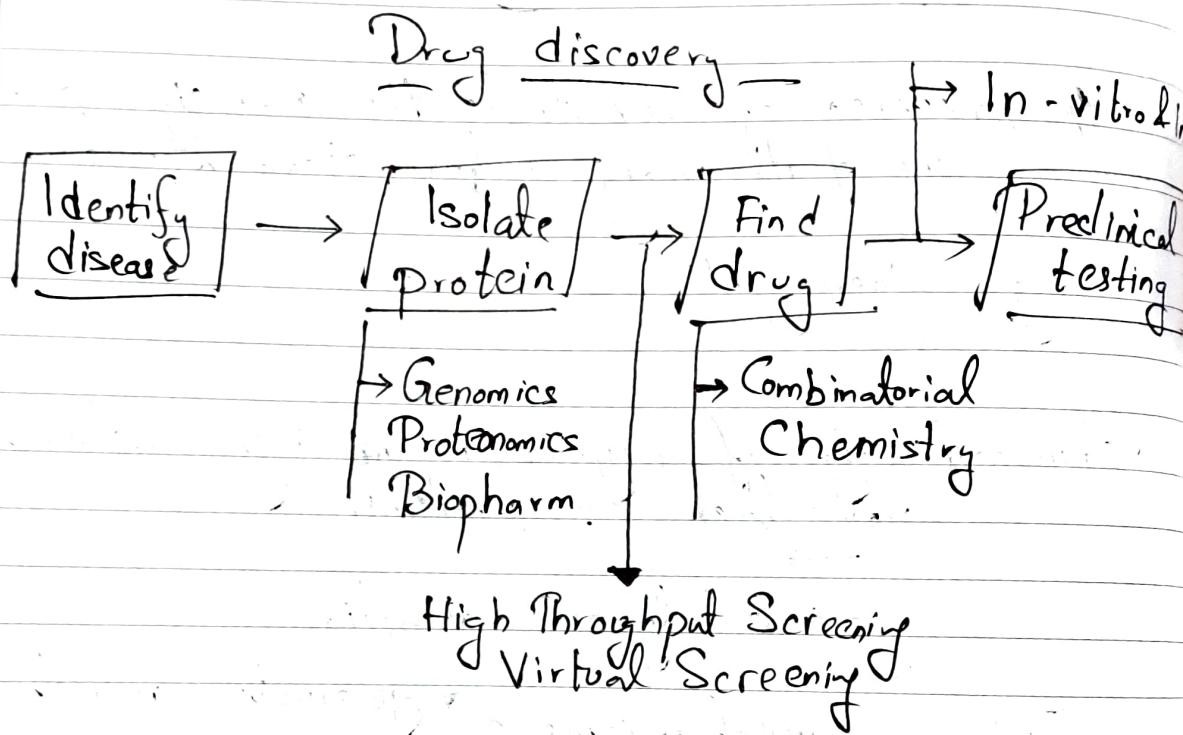
Metabolome → all chemicals in human body

Microbiome →

In-silico → In computers

In-vitro → in a lab

In-vivo → in the organism.



Repositioning / Reposing of drugs.

If a drug goes to different part of the body & causes some unwanted effects.

Forces in biomolecular system

→ London dispersion:

$$\text{Total energy} , U(\vec{R}) = \underset{\substack{\text{dist.} \\ \text{twist of atoms}}}{\text{Bond energy}} + \underset{\substack{\text{Angle energy} \\ \text{Lenard Jones}}}{\text{Angle energy}} + \underset{\substack{\text{Dihedral energy} \\ \downarrow}}{\text{Dihedral energy}} + \underset{\substack{\text{Nonbond energy} \\ \downarrow}}{\text{Nonbond energy}}$$

Analysis of DNA & RNA

classmate

5.9.22

Date _____

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Distinguishing animals by Phenotype.

Classification of living organisms by phylogenetic tree.

Last universal common ancestor.

Bacteria Archaea Eukarya

Prokaryotes

Eukaryotes

→ Unicellular, simple.

→ Multicellular, complex.

→ No nuclear membrane.

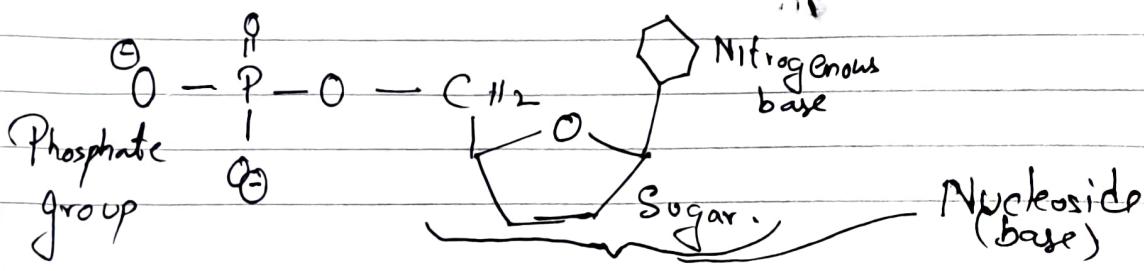
→ Nuclear membrane.

Cell → Nucleus → Chromosomes → DNA/RNA.

Chromosomes are present in nucleus of eukaryotic cell.

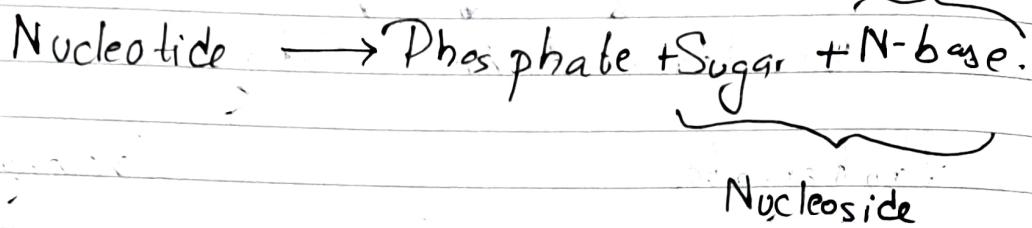
46 chromosomes in human beings.

Nucleotide: Nucleic acid monomeric unit.



Both DNA & RNA are bimeric polymers.

Nitrogenous base is a heterogeneous cyclic aromatic compound. (one C is replaced by N)



~~RNA~~ Nucleic acid → polymer of nucleotide.

Nucleobases

Pyrimidines

- ① Cytosine (C)
- ② Thymine (T) (DNA)
- ③ Uracil (U) (RNA).

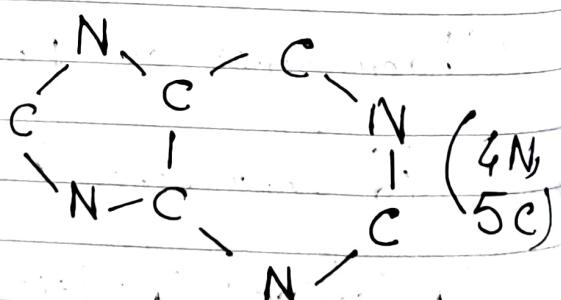
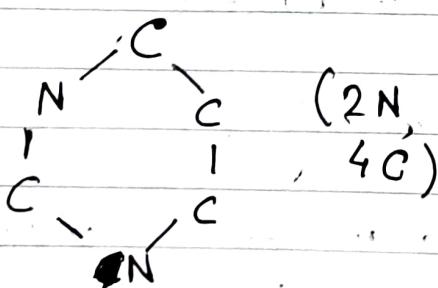
6-membered ring

Purines

- ① Adenine (A)
- ② Guanine (G).

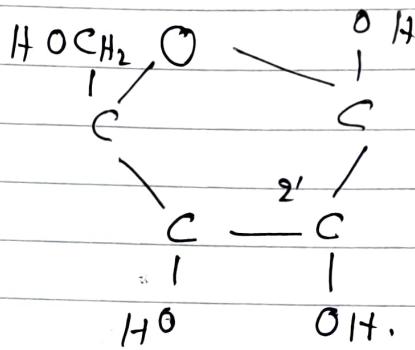
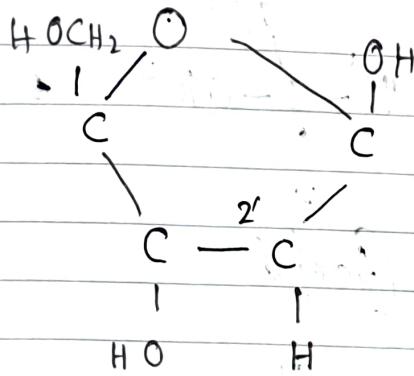
9-membered ring

Backbone structure.

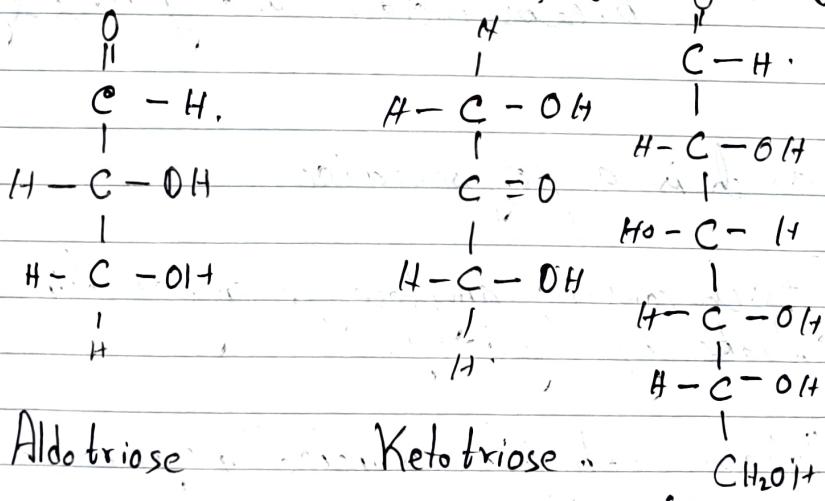


Pentose Sugars

Deoxyribose Ribose



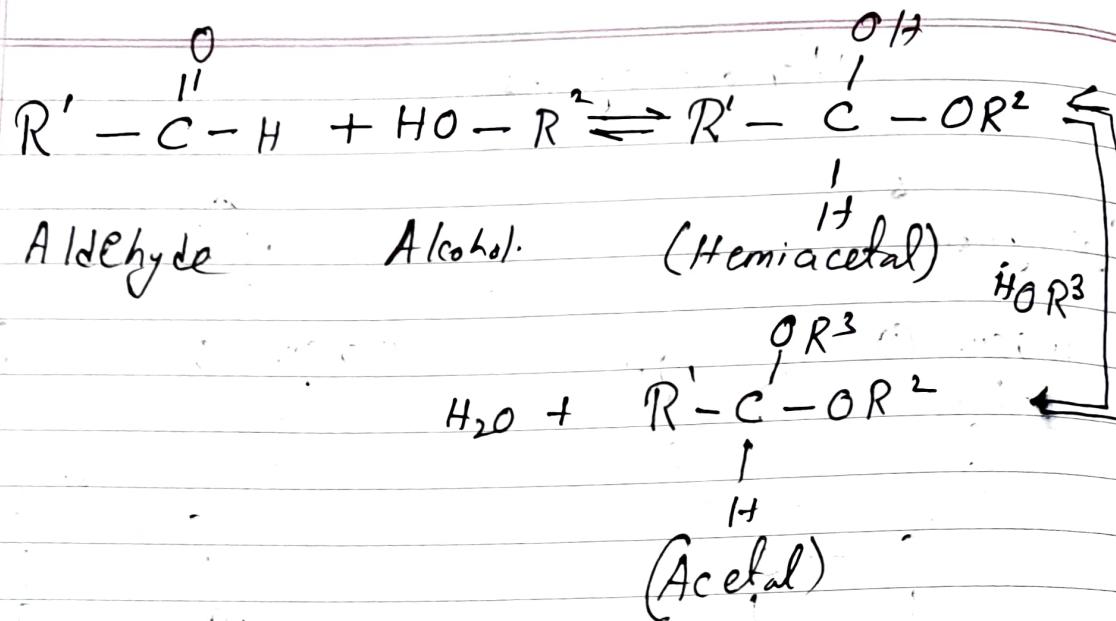
- ① Sugars are aldehyde or ketone.
- ② Sugars has a lot of $-OH$ groups. (poly-alcohol)



Glucose is an aldohexose.

Fuctose is a ketohexose. (sweetest.)

Sucrose \rightarrow disaccharide of glucose & fructose.)



$$\text{Lactose} \rightarrow \text{Galactose} + \text{Glucose}$$

$$\text{Trehalose} \rightarrow \text{Glucose} + \text{Glucose}$$

Lactase → Enzyme. (Works on lactose.)
breaks lactose into glucose & galactose.

Starch is a polysaccharide.

Starch contains amylose & amylopectin.
→ Broken by hydrolysis.

Cellulose → highly-branched polymer.

↳ Broken by cellulase into glucose molecule

Lignin → has a lot of organic chemicals

Hemicellulose → Pentose & Hexose.

Deoxyribonucleotides.

↳ Phosphate + Sugar ~~o~~ + N-base
(deoxyribo) (A, T, G, C).

When you have the prime, it is the backbone.

DNA supercoiling

$$\text{Linker N.} \quad \text{Superhelical density } \sigma = \frac{\Delta Lk}{Lk_0}$$

$$Lk = Tw + Wr$$

DNA is very strained.

All proteins need to be synthesized by the chromosomes.

DNA consists of exons & introns.

Intr

Exons do not contribute to anything. (Large segment)

Term that synthesizes proteins is called ~~intron~~ exons.

STR (Short Tandem Repeats).

↳ repeat sequence length: 2-9 base pairs/repeat.

VNTR (Variable Number of Tandem Repeats).

A gene is a section of DNA.

Cell → Nucleus → Chromosome → DNA → Gene.

A part of DNA that synthesizes a protein is gene.

Alleles are different of same gene.

Variant of gene.

e.g.- For a gene ACGT & SRTCA.

Allele 1: ACGTCAACGT

Allele 2: ACGTCACAACGT.

Allele 3: ACGTCACACAACGT

Homozygous



Heterozygous



Mendelian genetics: Punnett square

	B	b	b	b
B	BB	Bb	Bb	Bb
b	Bb	bb	b b	bb
b	Bb	b b	b b	bb

Certain features of alleles constitute the ~~space~~.

Protein synthesis

-
- The diagram illustrates the process of protein synthesis. At the top, a double helix represents DNA. An arrow points from DNA to mRNA, which is labeled "comes out from". Below mRNA, a ribosome is shown with arrows pointing to it from both DNA and mRNA. A third arrow points from the ribosome down to the bottom, where the text "Synthesis of protein" is written.
- ① Synthesis of mRNA
 - ② Movement of mRNA into cytoplasm
 - ③ Synthesis of protein.

mRNA → messenger RNA.
tRNA → translational RNA.

Codon → collection of 3 base pairs

Transcription → getting RNA from DNA.

Viral infections.

Virus have RNAs.

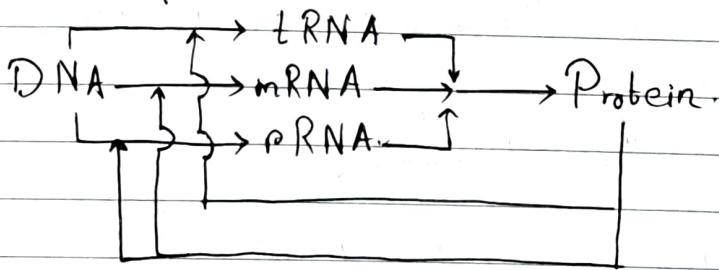
They enter the cells & do reverse - transcription.

They make a DNA & then undesirable proteins are created.

PCR → Polymerase chain reaction.

Identical twins are example of natural cloning.

DNA protein relation.



tRNA → responsible for choosing correct protein / amino acids required by the body in turn helping the ribosomes. (transfer sp. amino acid that are reqd.)

rRNA → component of ribosome.

mRNA → sign & translation of mRNA into protein.

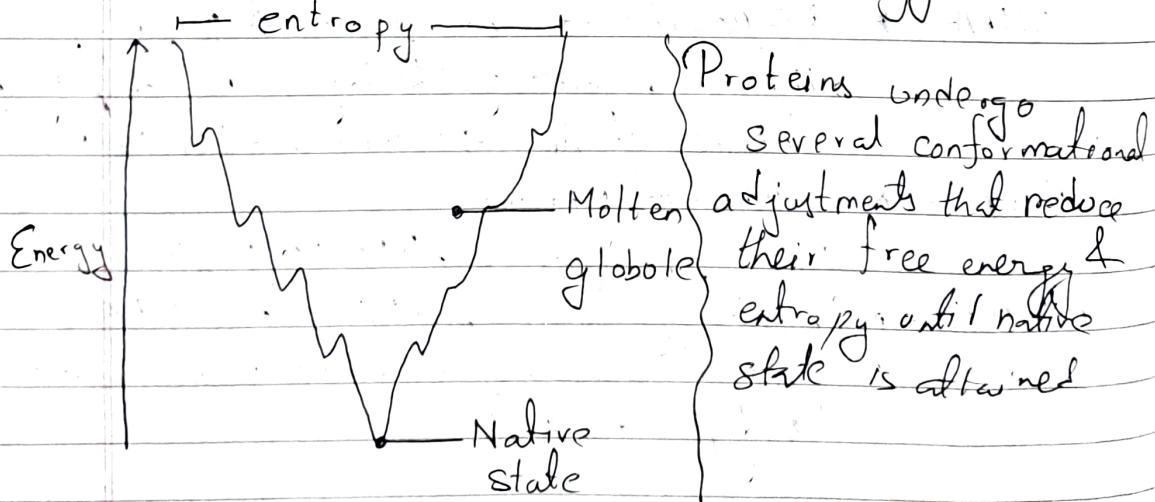
mRNA → transferring genetic material from DNA to ribosome & pass instructions about the type of protein.

Hydrophobic Collapse

- Folding is initiated by spontaneous collapse of the polypeptide into a compact state, mediated by hydrophobic interactions among non-polar residues.
- Water causes shielding effect on the non-polar residues.
- Water migrates towards non-polar residues and gets arranged / aligned.
- This causes dec. in entropy.
- Water inc. its entropy by folding & aggregating the non-polar residues.

Landscape theory

- Conformational adjustment of protein until it reaches lowest Gibbs energy.



Monosaccharides

Triose → D-Glyceraldehyde Aldhyde Ketone
Dihydroxyacetone.

Pentose → D-ribose D-ribulose.

Hexose → D-Glucose D-fructose
D-Galactose

Sucrose → fructose + Glucose

Lactose → Galactose + Glucose

^{water} ↗ Amylose → St. chain polymers of glucose. } starch
^{insoluble} ↗ 20% of starch.

^{water} ↗ Amylopectin → 25 glucose in chain
^{soluble} → con'td. betw. glycosidic - OH on one chain & 6 carbons on another glucose.

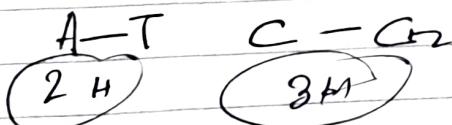
Cellulose → cotton & wood.

↳ long unbranched chain of D-glucose.

Lignocellulose → Hemicellulose.

→ encased in extensively cross-linked coating of lignin
→ found in wood surrounding clusters of microfibrils
→ branched polymers of pentose & hexoses.

Nucleotide → N-base
→ Phosphoric acid
→ Ribose & / Deoxyribose



Role of protein → catalysis

Polymerase Chain Reaction

1. Denaturation.

→ heating (addition) to separate the 2 strands of DNA molecule.

2. Annealing

→ Use of Primer

3. DNA Synthesis.

→ DNA polymerase (using nucleotides)

Primary — Amino acid sequence.
 Secondary — Manner of extension of polymer chain, due largely to H-bond betw. residue
 Tertiary — Folding, bending of polymer chain.
 induced by H, salt & disulfide bonds,
 hydrophobic & hydrophilic interactions.

Quaternary — How diff. polypeptide chains fit together; structure stabilized by same forces as tertiary structure.
 H-bonding of

1.5 Å α -helix → one which allows $-C=O$ & NH of its neighbor four units down the chain.

3.5 Å β -sheet → H-bonds of NH & $C=O$.

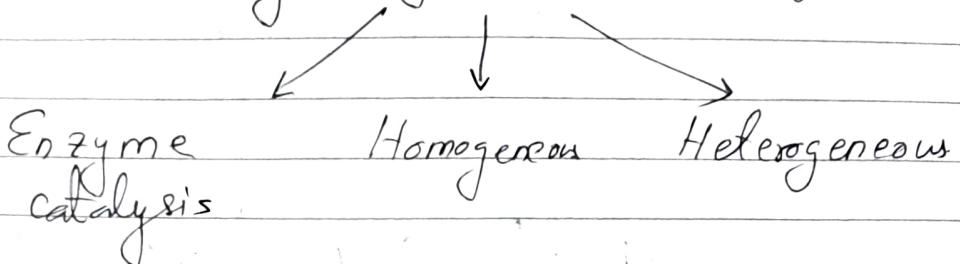
Analysis of Enzymes

classmate

Date 10.10.22

Page _____

- Catalyst — every enzyme is a catalyst.

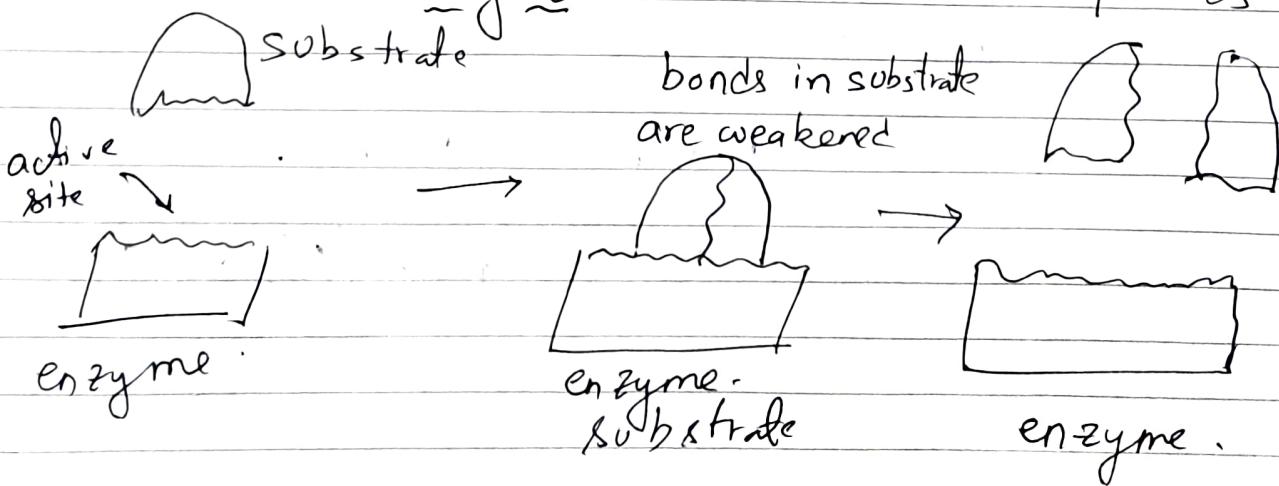


- Proteins (all enzymes are proteins)
 - Majority of proteins of any body are enzymes.
- Highly specific.

Classification of enzymes

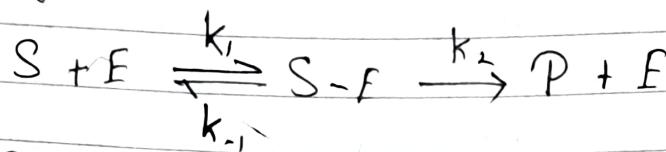
Oxidoreductases	— Transfer of e ⁻ s (H atom)
Transferases	— Group transfer rxns.
Hydrolyases	— Hydrolysis rxn.
Lyases	— Cleavage of C-C, C-O, C-N
Isomerases	— Transfer of groups within molecules
Ligases	— Formation of C-C, C-S, C-O or C-N bonds by condensation

Working of enzymes



Understanding enzyme kinetics: Michaelis-Menten model.

Enzymes do not follow the law of mass action.



$$\frac{d[S]}{dt} = k_{-1}[S-E] - k_1[S][E]$$

$$\frac{d[E]}{dt} = (k_{-1} + k_2)[S-E] - k_1[S][E]$$

$$\frac{d[S-E]}{dt} = k_1[S][E] - (k_2 + k_{-1})[S-E]$$

$$\frac{d[P]}{dt} = k_2[S-E]$$

None of
these
are
applicable

The system as a whole will not follow law of mass action but the individual rxns will follow law of mass action.

We cannot determine $[S-E]$.

We want to get $P = f([P] \text{ or } [S])$

Equilibrium approximation

Assumption: Substrate is in instantaneous equilibrium with the complex.

$$V = \frac{V_{\max} [S]}{K_1 + [S]}$$

$$V = k_2 [S - E] \quad \xrightarrow{\text{rate constant}}$$

$$k_1 [S][E] = k_{-1} [S - E]$$

$$[S - E] = \frac{k_1}{k_{-1}} [S][E] = \frac{1}{K_1} [S][E]$$

$$V = k_2 \cdot \frac{1}{K_1} \cdot [S][E] \quad \hookrightarrow \text{eqm. const.}$$

To get rid of E , let's do an enzyme balance,

$$[E](t) + [E - S](t) = [E](t=0)$$

$$[E]_0 = [E]_0 + [E - S]$$

$$\Rightarrow [E]_0 = [E] + \frac{1}{K_1} [S][E]$$

$$\Rightarrow [E]_0 = \left(1 + \frac{[S]}{K_1}\right) [E] \Rightarrow [E] = \frac{[E]_0}{1 + [S]/K_1}$$

$$\Rightarrow [E] = \frac{k_1 [E]_0}{K_1 + [S]}$$

$$V = \frac{k_2 [E]_0 [S]}{K_1 + [S]} = \frac{V_{\max} [S]}{K_1 + [S]}$$

↑ rate constant
 ↓ eqm. constant.

$$V_{\max} = k_2 [E]_0$$

$$\frac{d[S]}{dt} = -k_2 \frac{[E]_0 [S]}{K_1 + [S]} \cdot \left(1 + \frac{[E]_0 K_1}{(K_1 + [S])^2} \right)$$

Vari Steady State Approximation

Assumption: Rate of formation & breakdown of the complex are equal at all time

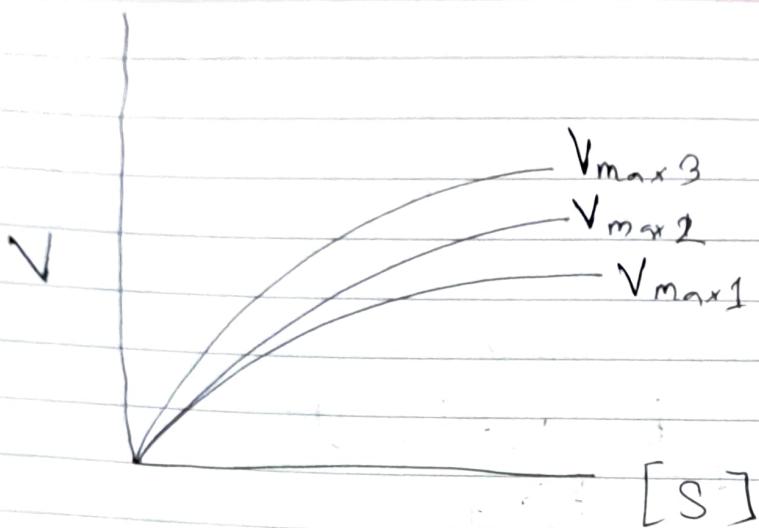
$$V = \frac{d[P]}{dt} = -\frac{d[S]}{dt} = \frac{V_{\max} [S]}{K_M + [S]}$$

$$k_1 [S][E] = k_{-1} [S-E] + k_2 [S-E]$$

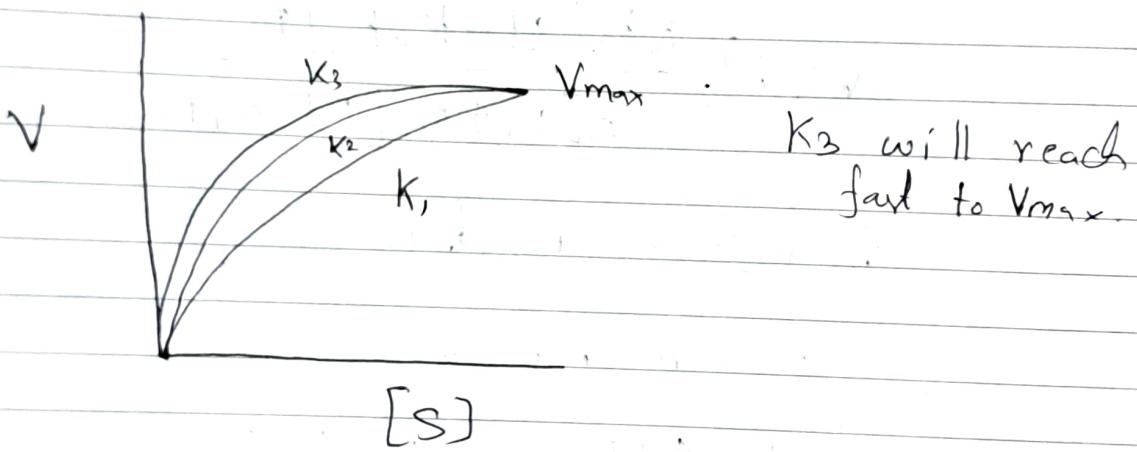
$$\Rightarrow [S-E] = \frac{k_1}{(k_{-1} + k_2)} [S][E] = \frac{1}{K_M} [S][E]$$

$$V = \frac{k_2}{K_M} [S][E] = \frac{k_2 [E]_0 [S]}{K_M + [S]} \quad [K_M = \frac{k_{-1} + k_2}{k_1}]$$

$$V = \frac{V_{\max} [S]}{K_M + [S]}$$

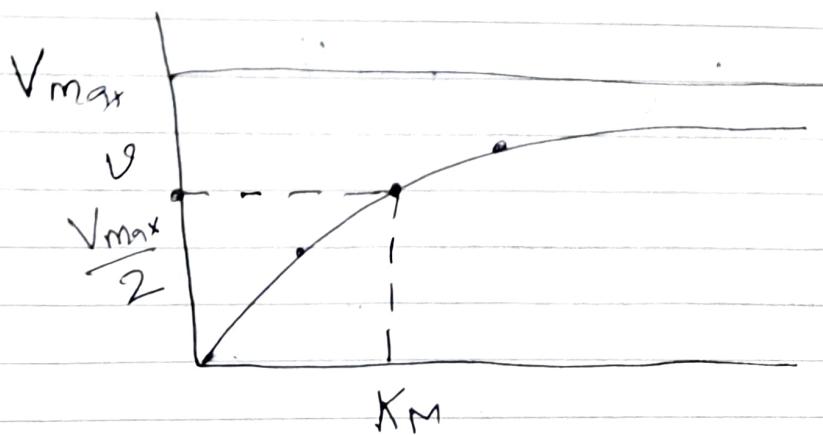


For different K_m .



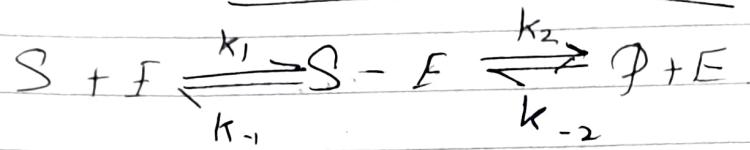
Determination of kinetics parameters.

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \frac{1}{[S]}$$



larger value
of K_m
↓
rise will
be slow.

Reversible rxn. Scheme



$E_g m$: approximation.

$$V = [E]_0 \frac{k_1 k_2 [S] - k_1 k_{-2} [P]}{k_1 [S] + k_{-1}}$$

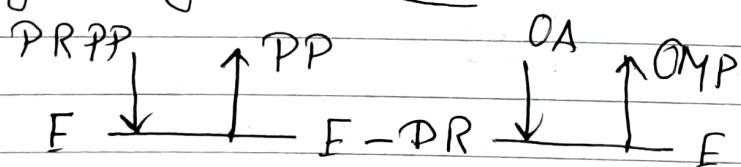
Dev'nt
Int'
for exam

Quasi steady state approximation

$$V = [E]_0 \frac{k_1 k_2 [S] - k_{-1} k_{-2} [P]}{k_1 [S] + k_{-2} [P] + k_{-1} + k_2}$$

Biomolecular reactions

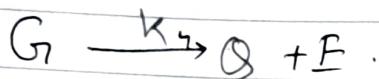
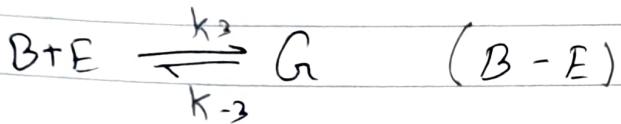
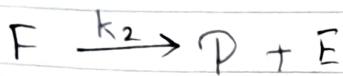
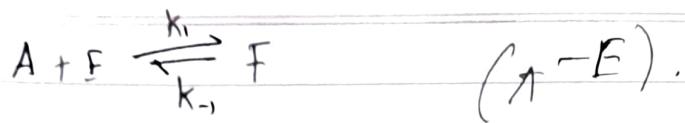
1. Ping-pong mechanism



→ Substrate are designated A, B, C & D in order they bind to the enzyme.

→ Products are designated P, Q, R & S in order they leave the enzyme.

→ Stable enzyme forms are designated E, F, G with E being the free enzyme.



Quasi Steady State Exp.

$$V = \frac{k_1 k_2 k_3 k_4 [E]_0 [A][B]}{k_1 k_2 [A] + (k_{-1} + k_2) k_3 k_4 [B] + k_1 k_3 (k_2 + k_4) [A][B]}$$

$$V = \frac{(k_1 k_2 k_3 k_4 [E]_0 C)[A]}{((k_1 + k_2) k_3 k_4 C + (k_1 k_3 (k_2 + k_4) C + k_1 k_2) [A])}$$

$$V = \frac{\left[\frac{k_1 k_2 k_3 k_4 [E]_0 C}{k_1 k_3 (k_2 + k_4) C + k_1 k_2} \right] [A]}{\left[\frac{(k_{-1} + k_2) k_3 k_4 C}{k_1 k_3 (k_2 + k_4) C + k_1 k_2} \right] + [A]}$$

\downarrow
 K_M

Ordered mechanism.



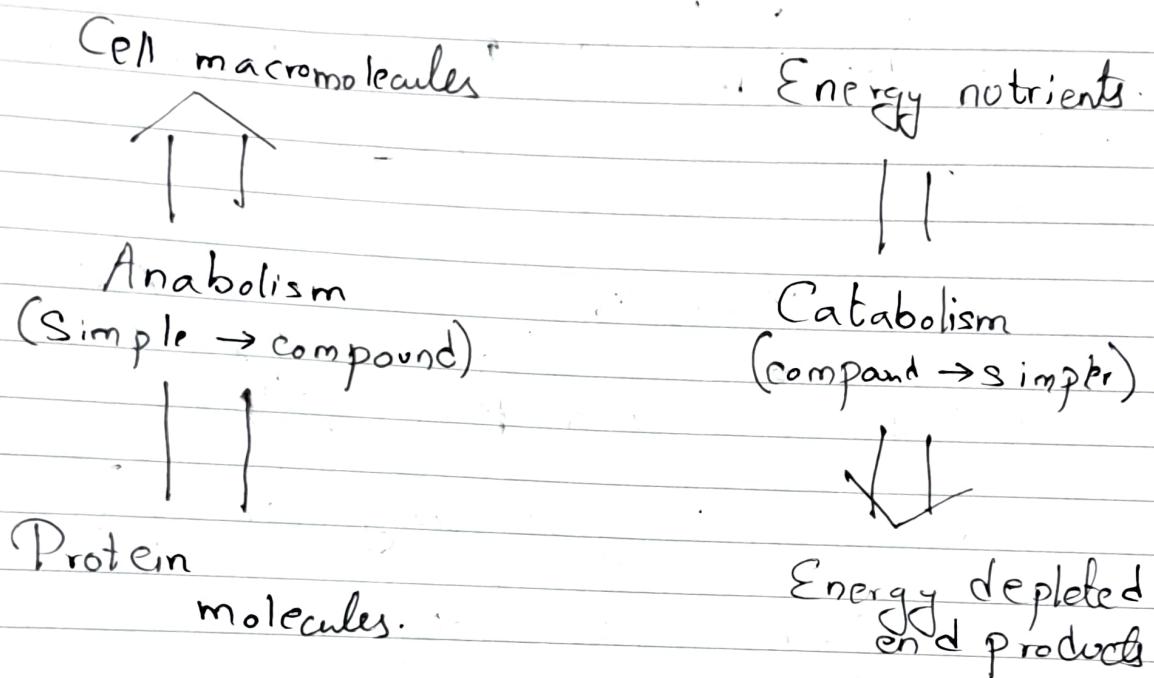
Analysis of Metabolism

classmate

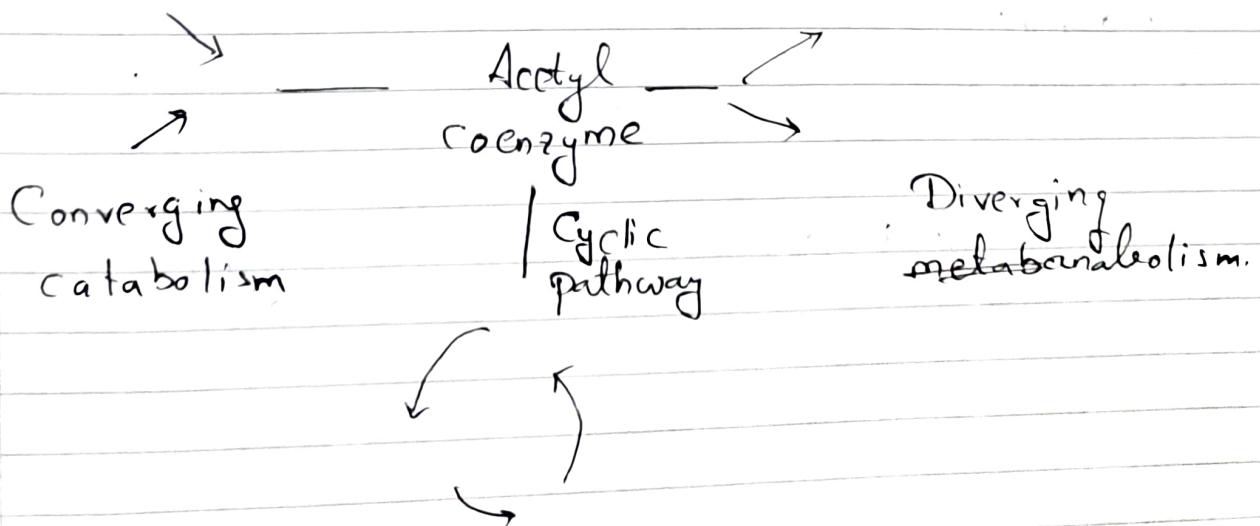
Date 31.10

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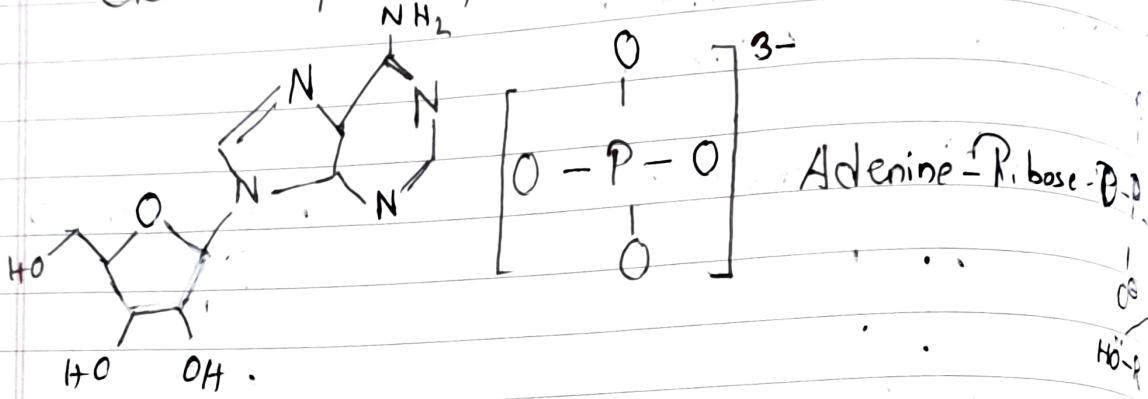
Chemical Process → Metabolite
→ Metabolism.
Collection → Metabolome



Human's metabolism is for energy changes.



Generation/consumption of energy. - Phosphorylation



Metabolite.

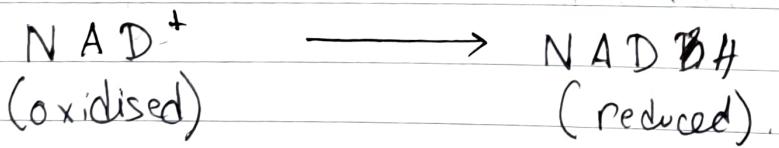
ATP.

+ Energy



A sp. that gives away $[\text{P}-\text{O}]$

Oxidation / Reduction

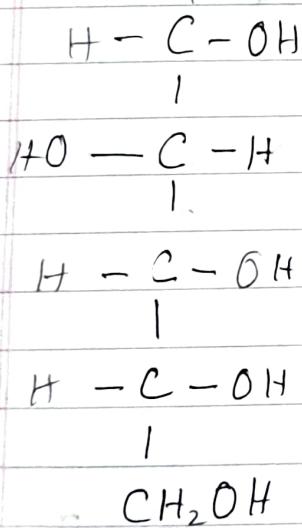
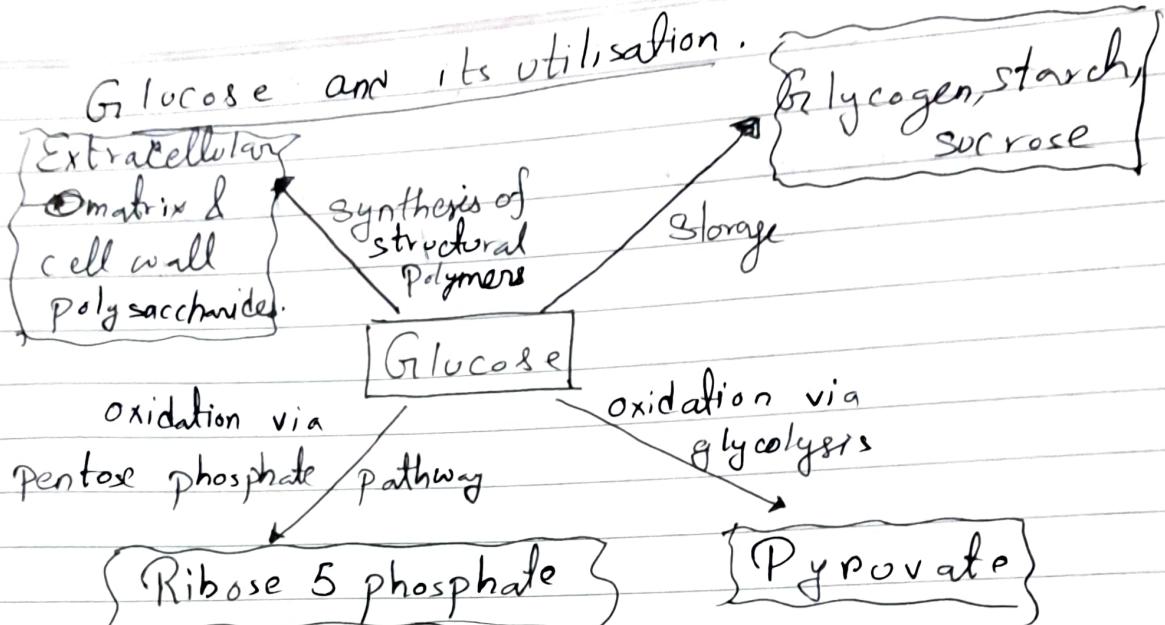


Nicotinamide

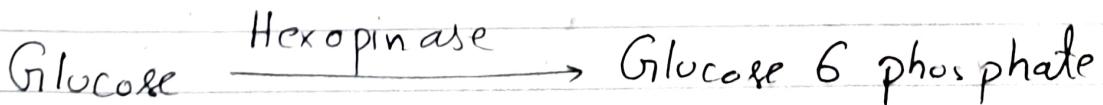
Adenine

Dinucleotide.

A sp. that can give away hydrogen

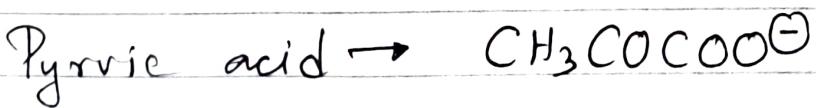


Glucose Molecule



Add phosphate & release energy.

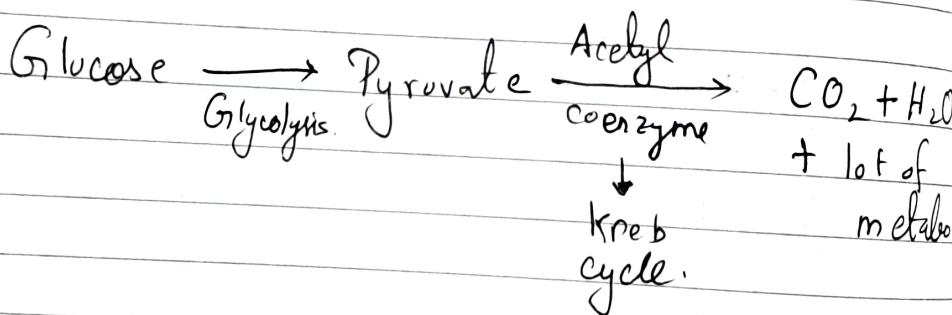
Simplest molecule \rightarrow Pyruvate



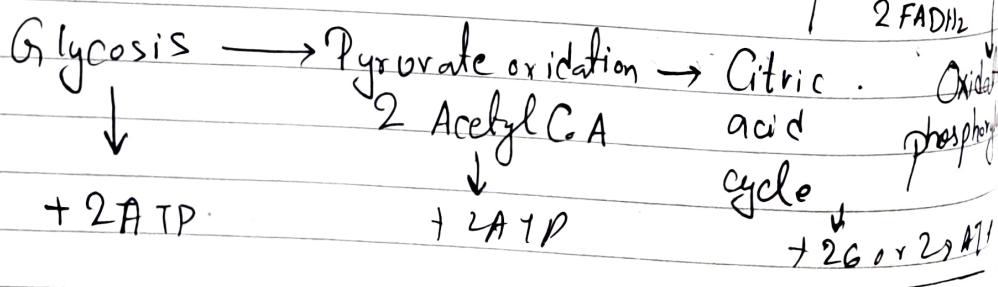
Acetyl coenzyme formation

Coenzyme → not a true enzyme
but helps in catalysis of

Citric acid / Krebs / TCA cycle.



Overall Energy gen.



30 - 32 molecules are formed ~~as for~~
Single glucose molecule.

Every sugar can be converted to glucose.

Lack of glucose & inefficiency of glucose processing

Plan A Ketone Bodies.

 ↘
Generate [Carbs
energy Fat
Proteins]

Plan B [Alternative fuel
source]

Produced by Liver mitochondria → Physiological states

Using Acetyl CoA → Pathological states.

Liver

→ Blood Majority of Cells

Reconverted into Acetyl CoA

↓
Mitochondria

Produce ATP

Acetoacetate β -Hydroxybutyrate Acetone.



Ketones too give us energy when reqd

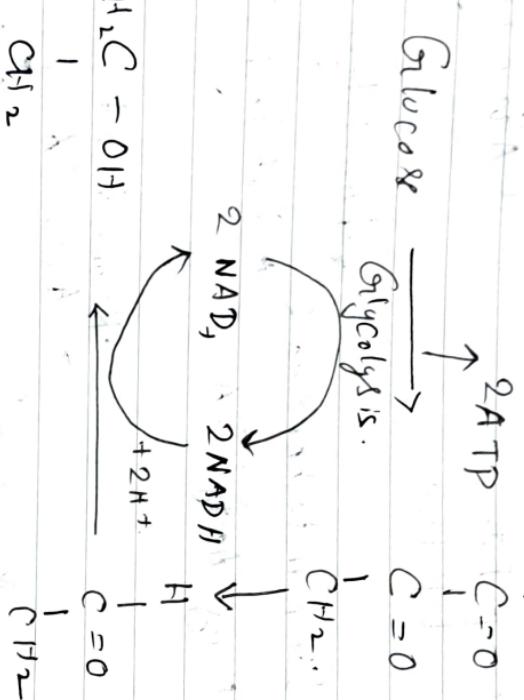
But pH is diff. from routine molecules.

All Rns. depend on certain pH. When pH changes rxn. rate changes.

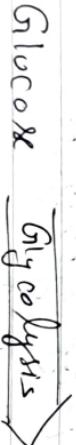
In diabetes, glucose is less & hence,
pH changes. \Rightarrow

Pyruvate utilisation.

a. Alcohol fermentation.



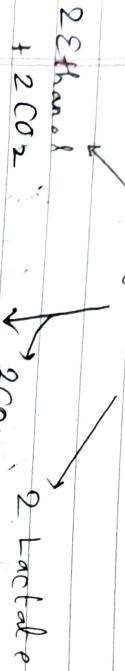
b. Lactic acid fermentation,



Glucose

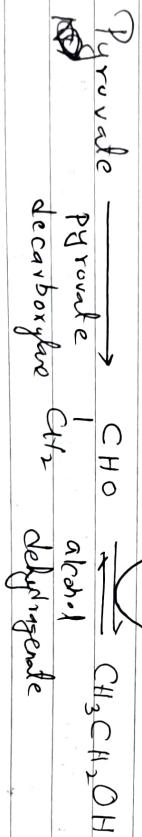
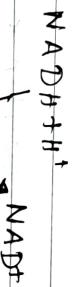


2 Pyruvate



2 Acetyl CoA

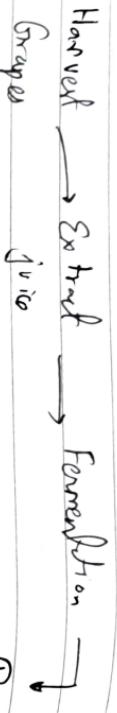
Krebs cycle



Congeners of ethanol.

- ↳ Trehalose; Melatonin; Tyrosol;
- Serotonin; Phenylethanol; Hydroxyresol

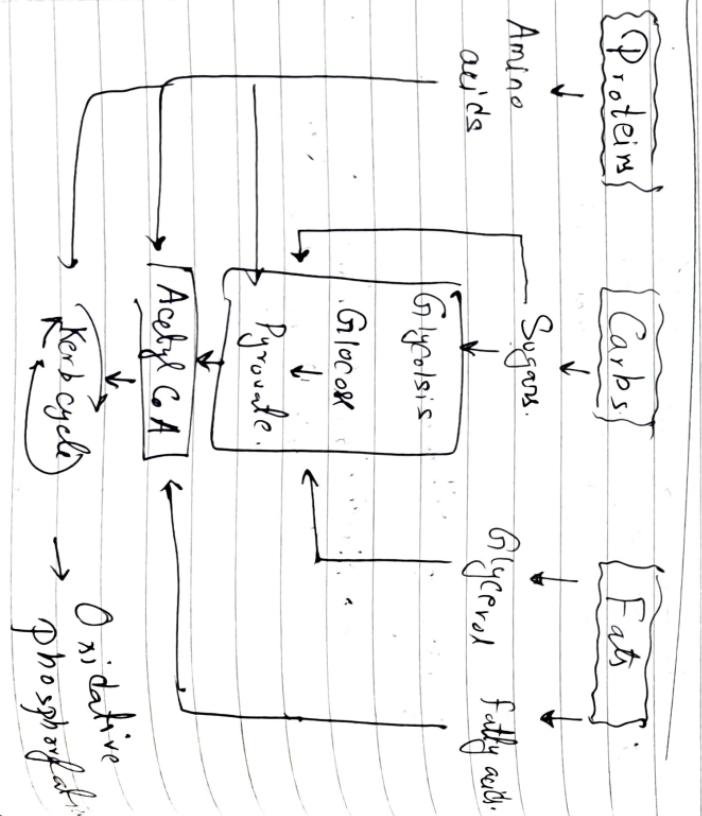
White v/s Whiskey v/s Vodka.



Consume ← Bottling ← Aging ← Filtration.

Bourbon making process.

Cooker → Fermentation → Column
Bourbon ← Condenser ←



Raw material of vodka \rightarrow sugar molasses.

Modeling strategy -

- Interaction based network
- Describe network connectivity
- Assume stationarity
- Describe the flow of information.
- Neglect stoichiometry.

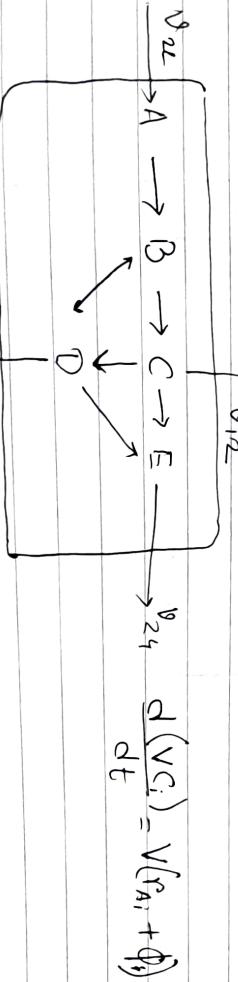
Stoichiometric models

- Assume stationarity
- Describe intracellular mass fluxes @ steady state.
- Describe fundamental cellular biochemistry.

Dynamic models

- Based on ODE
- Based on kinetic information.
- Give temporal changes

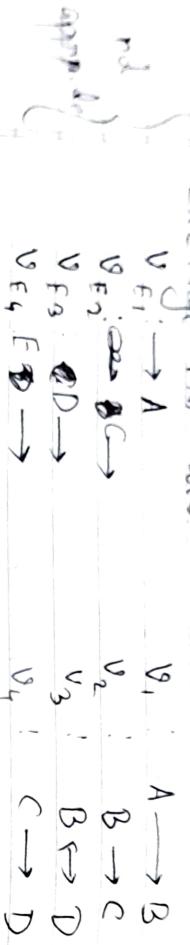
Metabolic flux analysis



$$V = \frac{V_{max} [A]}{K_m + [A]} ; \quad \frac{d [CV_i]}{dt} = V(r_i + \phi_i).$$

Metabolites A, B, C, D, E

Exchange rate:



v_i → intracellular fluxes
 v_{f_i} : exchange fluxes

$$\frac{d[A]}{dt} = v_{E_1} - v_1 \quad \frac{d[B]}{dt} = v_1 - v_2 - v_3$$

$$\frac{d[C]}{dt} = v_2 - v_4 - v_{E_2} \quad \frac{d[D]}{dt} = v_3 + v_4 + v_6 - v_{E_3}$$

$$\frac{d[E]}{dt} = v_5 + v_6 - v_{E_4}$$

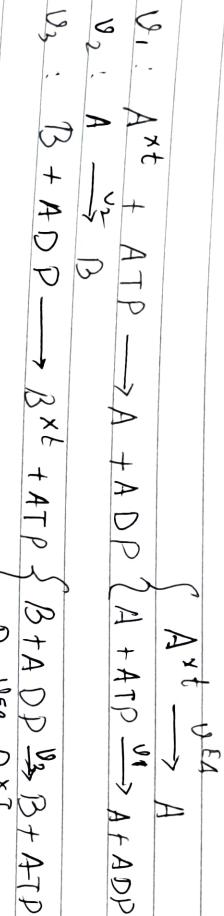
$$\frac{d}{dt} \begin{bmatrix} [A] \\ [B] \\ [C] \\ [D] \\ [E] \end{bmatrix} = \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \end{bmatrix}$$

$$\begin{bmatrix} v_{E_1} \\ v_{E_2} \\ v_{E_3} \\ v_{E_4} \\ v_{f_1} \\ v_{f_2} \\ v_{f_3} \\ v_{f_4} \end{bmatrix}$$

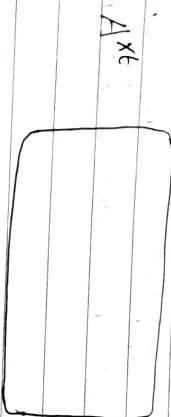
S : Stochiometric matrix.
 Σ : Flux distribution array

Q: Steady state : $Sv = 0$

Extracellular metabolites: $A^{xt}, B^{xt}, C^{xt}, D^{xt}$



1. Draw network for above mentioned system.



2. Write flux balance equations

3. Develop diff. matrices & write the relationships among them @ steady state.

4. Carry out deg. of freedom analysis.

5. Generalise the D.O.F. analysis hence due to come up with rules for determinacy of str.

Common obj

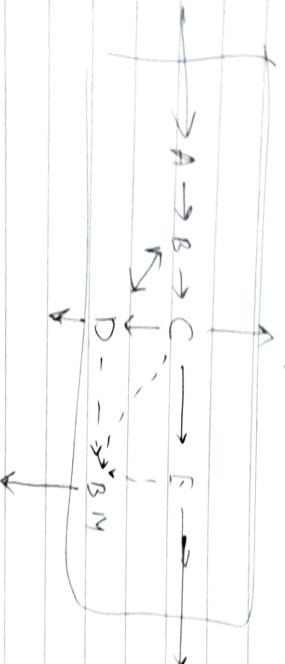
→ Maximisation of biomass yield

→ Maximisation of growth rate

→ Maximisation of ATP yield per flux.

→ Min. of glucose consumption

Max of biomass



1. Penicillin

2. Heterofermentation of lactic acid bacteria.

Dynamical flux balance analysis
Glycolytic oscillation.

Glucose

n S_n

Glucose G-6-P

Fischer ester

fructose