Vivekanand College,Kolhapur (Empowered Autonoums)

Department of Microbiology

PPT Bank

(2018-2023)

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Applied Microbiology.

Presented by

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B) Water Microbiology:

a) Sources of microorganisms in water.
b) Fecal pollution of water, Indictors of fecal pollution of water –E. coli
c) Routine Bacteriological analysis of water.
1) SPC & 2) Tests for coliforms -
i. Qualitative-Detection of coliforms - Presumptive test, Confirmed Test, Completed test.
Differentiation between coliforms - IMViC test, Eijkman test.
ii. Quantitative – MPN, Membrane filter technique
d) Municipal water purification process and its significance.

- Sources of Microorganisms In water:

Normal flora of water is classified in to two types : 1) Typical Flora

2) Atypical Flora

Typical Flora : found in certain environment

Typical Flora is as follows :

A) psychrophilic organisms-*Aeromonas, Alcaligens, Arthrobacter, corynebacterium, Proteus, streptococcus &Vibrio*

B) Thermophilc bacteria -*Bacillus* and *Thiobacillus Spp.*

C) Anerobes: *Clostridium, And Desulfovibrio*

*D)*Iron mine areas *-Thiobacillus ferroxidence*

- Atypical flora: found in any aqueous environment

  Example : *Micrococcus, Flavobacterium, chromobacter, pseudomonas, Bacillus, streptomyces, Nocardia, algae and protozoa*

  Bacteriophages,animal & plant viruses,yeast like *cryptococcus* & mold like *Aspergillus, Penicillum,Mucor & their spores* also present

Fecal pollution of water:

Major sources of fecal pollution:

1. Treated Sewage Effluent
2. urban storm water
3. rural storm water
4. wild type refuges

Microorganisms found in fecaly polluted water:

A)Pathogenic as well as non-pathogenic bacteria found *salmonella,shigella,vibrio,and leptospira*

B)Viruses -*hepatitis B, and polio* virus

C) protozoa -*Entamoeba histolytica*

Indicators of fecal pollution-

Definition of indicator -An organism whose presence or absence in water indicates water quality.

Best indicator is the organism which full fill following requirement.

1.it should be consistently & exclusively associated with the source of pathogen

2.the indicators should be harmless

3.it should be present in accurate no. to provide accurate density estimate.

4.it should survive longer than pathogen

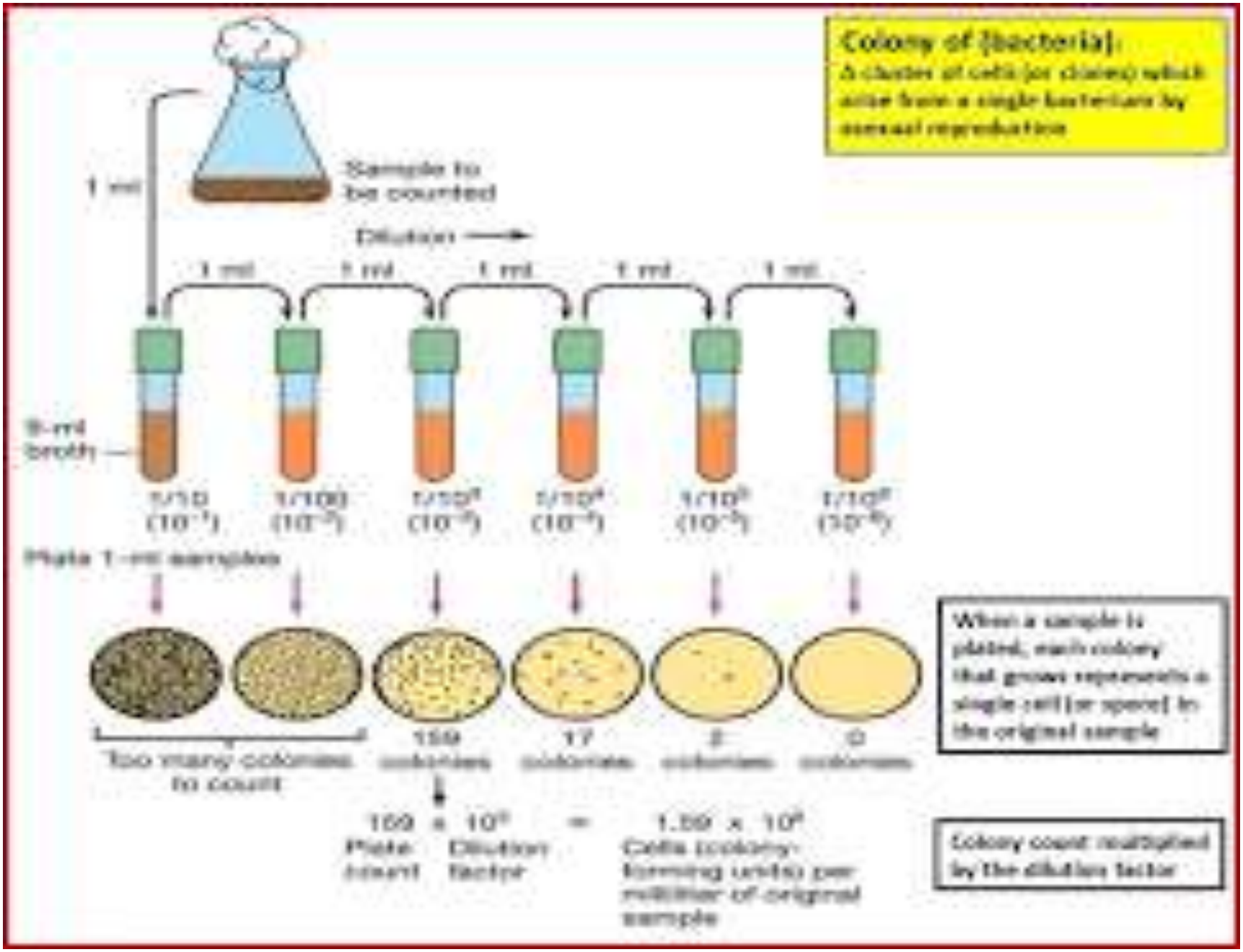
5.it should be quantity by reasonably feasible and inexpensive method with considerable accuracy and specificity.

Best indicator

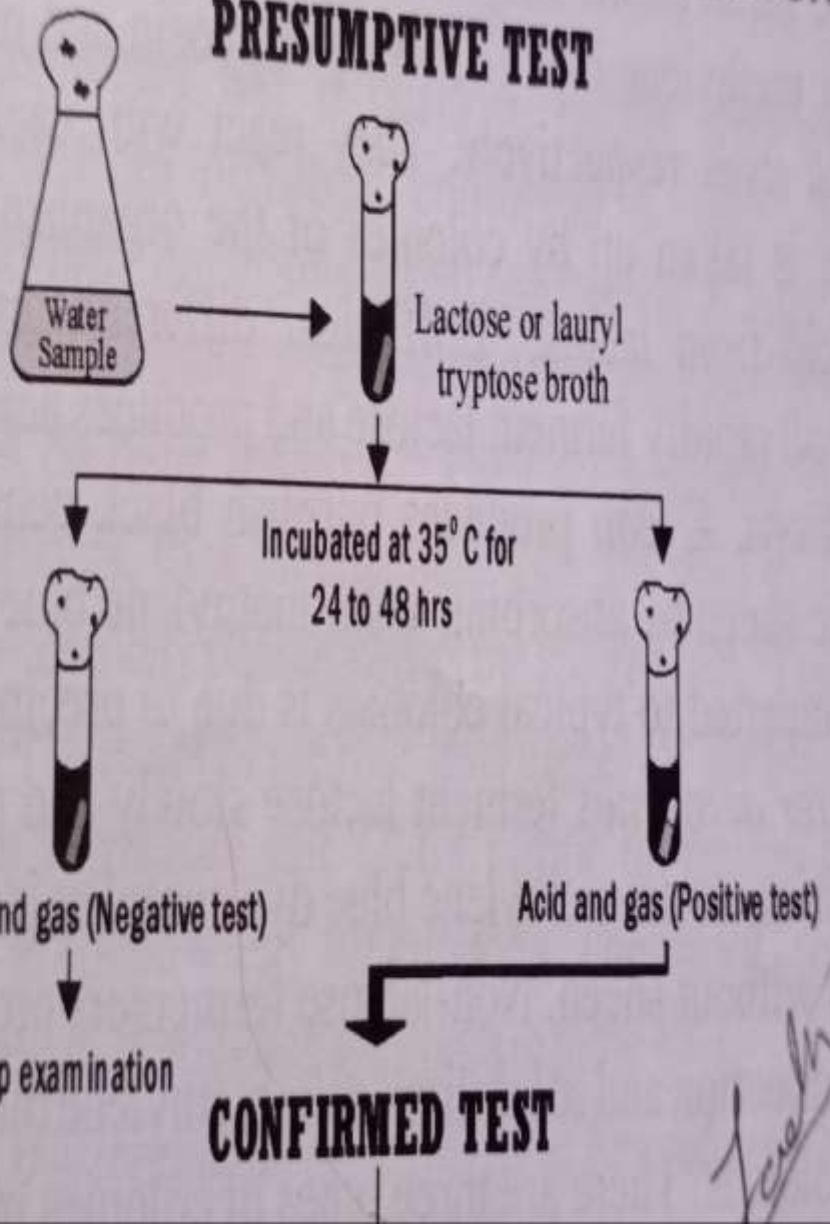
Escherichia coli

Streptococcus fecalis

Bifidobacterim


Colony of [bacteria]:
1 ml
Sample to be counted
Dilution
1 ml
1 ml
1 ml
1 ml
1 ml
9-ml broth
1/10 (10^{-1})
1/100 (10^{-2})
1/1000 (10^{-3})
1/10^4 (10^{-4})
1/10^5 (10^{-5})
1/10^6 (10^{-6})
Plate 1-ml samples
Too many colonies to count
159 colonies
17 colonies
2 colonies
0 colonies
When a sample is plated, each colony that grows represents a single cell [or spore] in the original sample
159 × 10^3 = 1.59 × 10^5
Plate count
Dilution factor
Cells (colony-forming units) per milliliter of original sample
Colony count multiplied by the dilution factor

Endo agar and Brilliant Green Lactose Bile



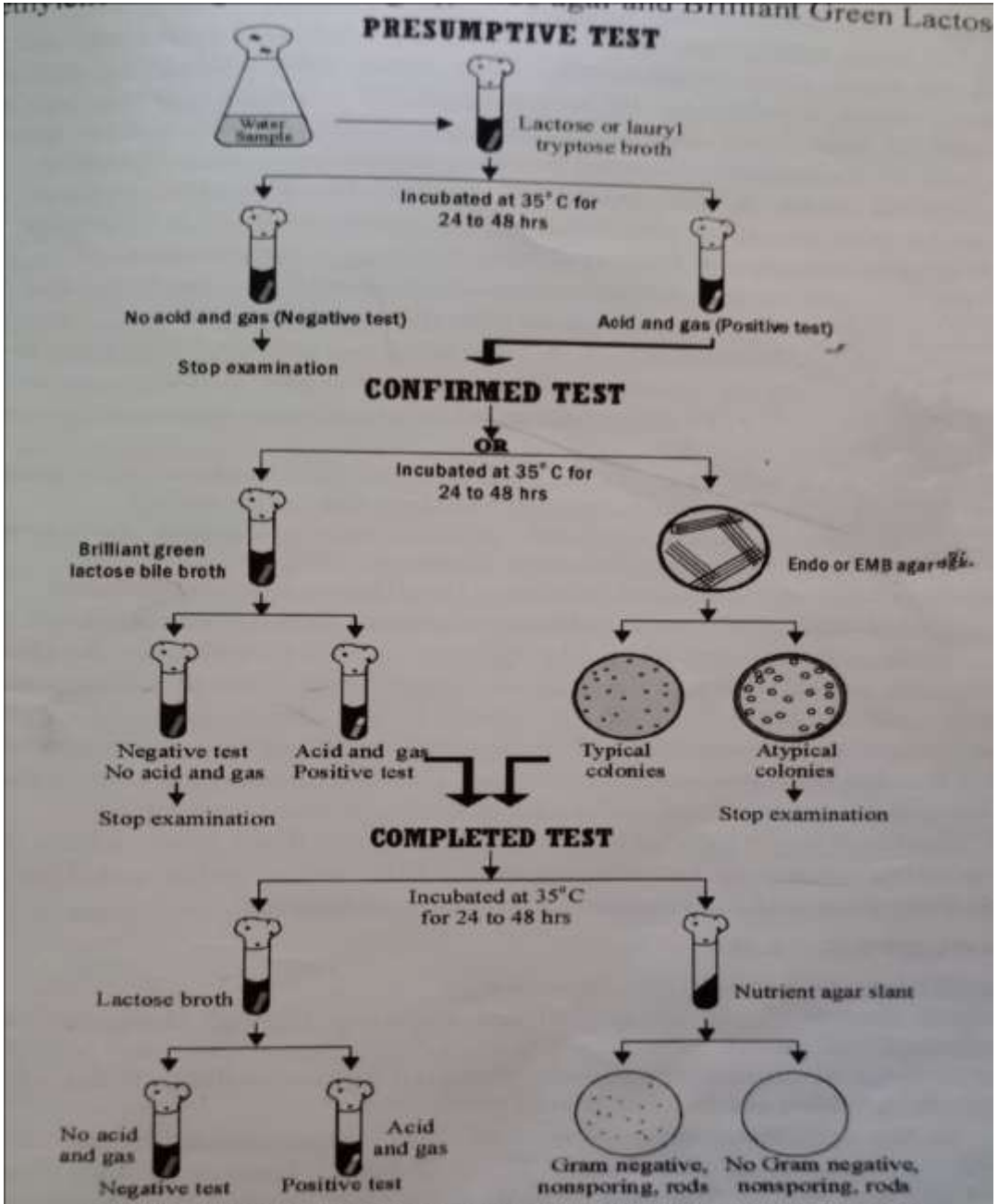


Fig .10.1: General scheme for determination of coliforms in water

IMViC test

I-Indol production test

M-Methyl red test

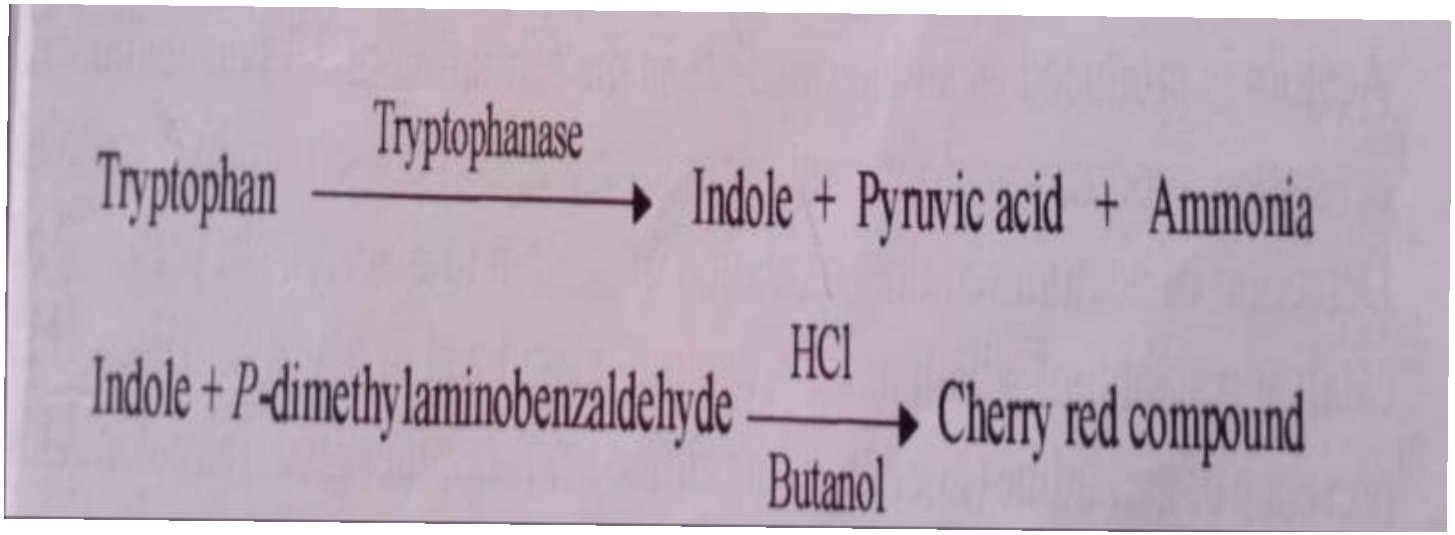
V-Voges Proskauer test

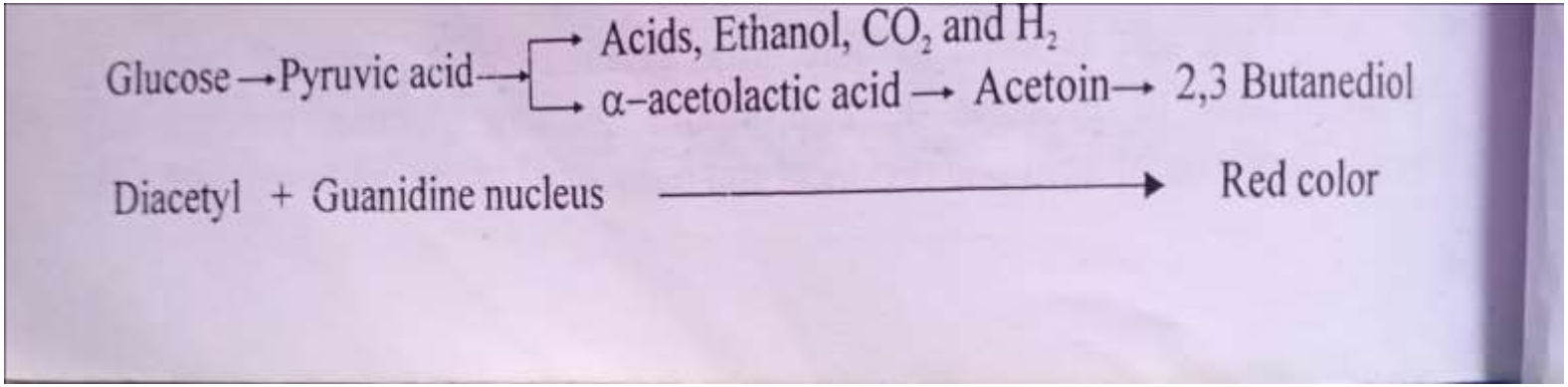
C-Citrate utilization test

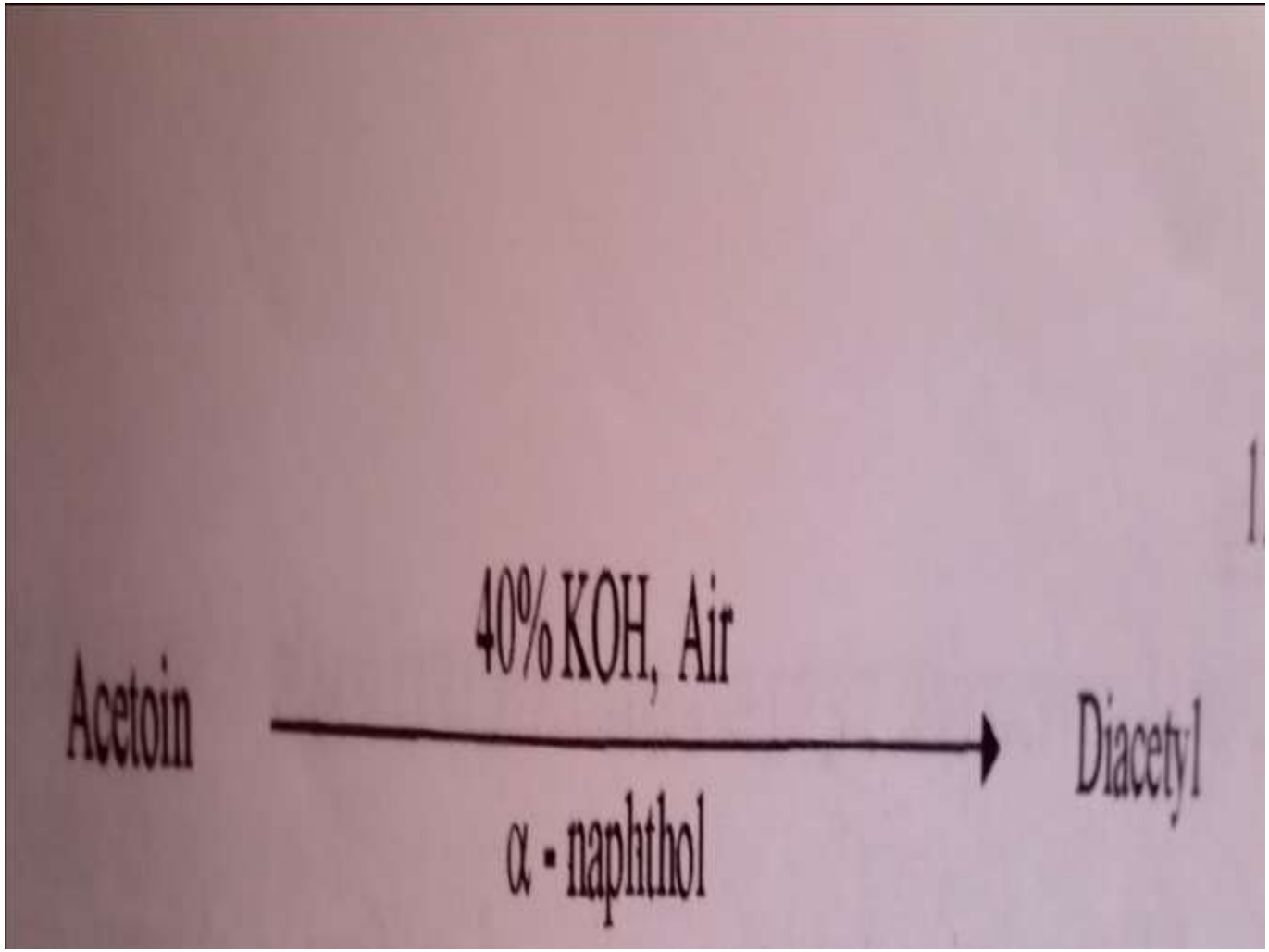
Named coined by **Parr**

Organism	Source	I	MR	VP	C
E. Coli	Feces	+	+	-	-
E. aerogenes	Soil /feces	-	-	+	+

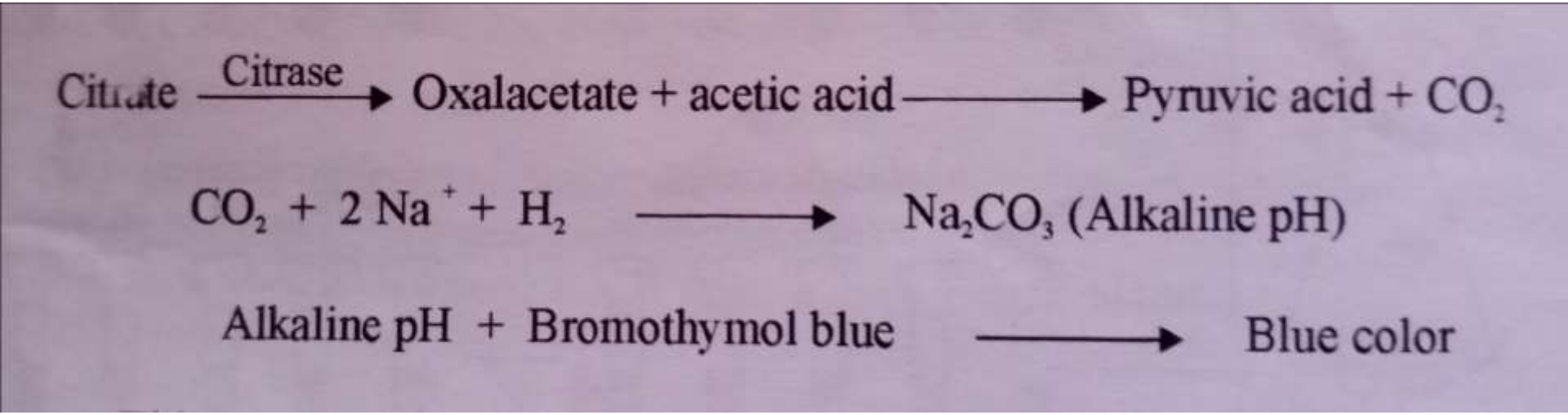
Indol production test




Glucose → Pyruvic acid → Acids, Ethanol, CO_2 and H_2
Pyruvic acid → α–acetolactic acid → Acetoin → 2,3 Butanediol
Diacetyl + Guanidine nucleus → Red color


40% KOH, Air
Acetoin
Diacetyl
α - naphthol

Citrate utilization



B. Quantitative Test for coliforms

1. Most Probable Number
2. Membrane Filter Technique

indicate positive result. The number of

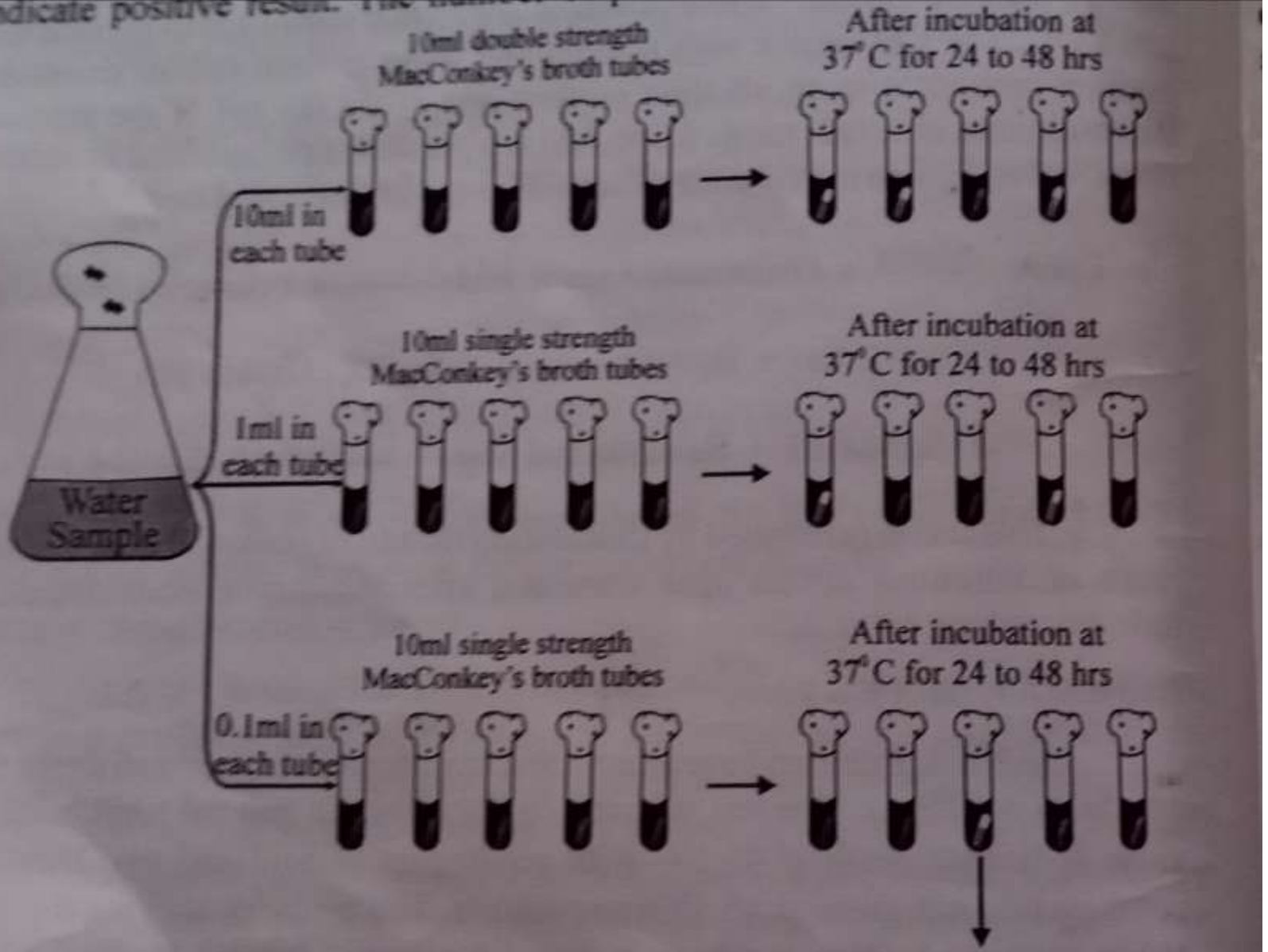


Fig .10.2: MPN procedure

Membrane Filter technique

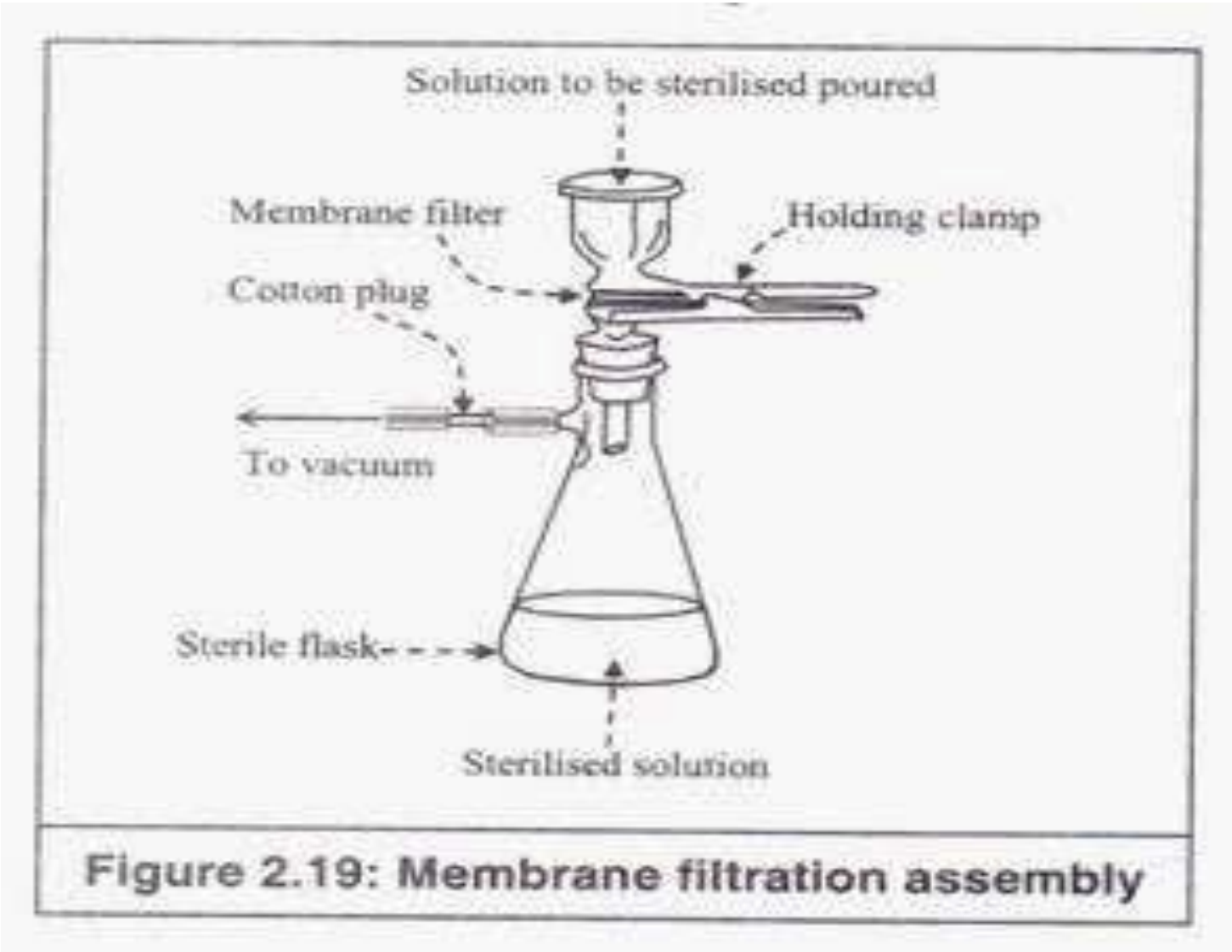


Figure 2.19: Membrane filtration assembly

- Municipal water purification process:

  A. Sedimentation
  B. Filtration
  C. Disinfection

HCl and HOCl.

$$Cl_2 + H_2O \longrightarrow HCl + HOCl$$

HOCl (hypochlorous acid) is very unstable and therefore immediately decomposes to release nascent oxygen.

$$HOCl \longrightarrow HCl + O^* \quad \text{(Nascent oxygen)}$$

This nascent oxygen is

Assignment

Q. 1-Define Coliform and describe test for Coliform In bacteriological examination of water

2.Explain municipal purification process

Q.2-short answer question

Confirmed test

Fecal pollution of water

- REFERENCE:
- Ananthanarayan & Panikar’s Textbook of Microbiology
- Pelczar Microbiology
- Handbook of water and wastewater Microbiology
- Text book of Microbiology

FATTY ACID OXIDATION

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Introduction

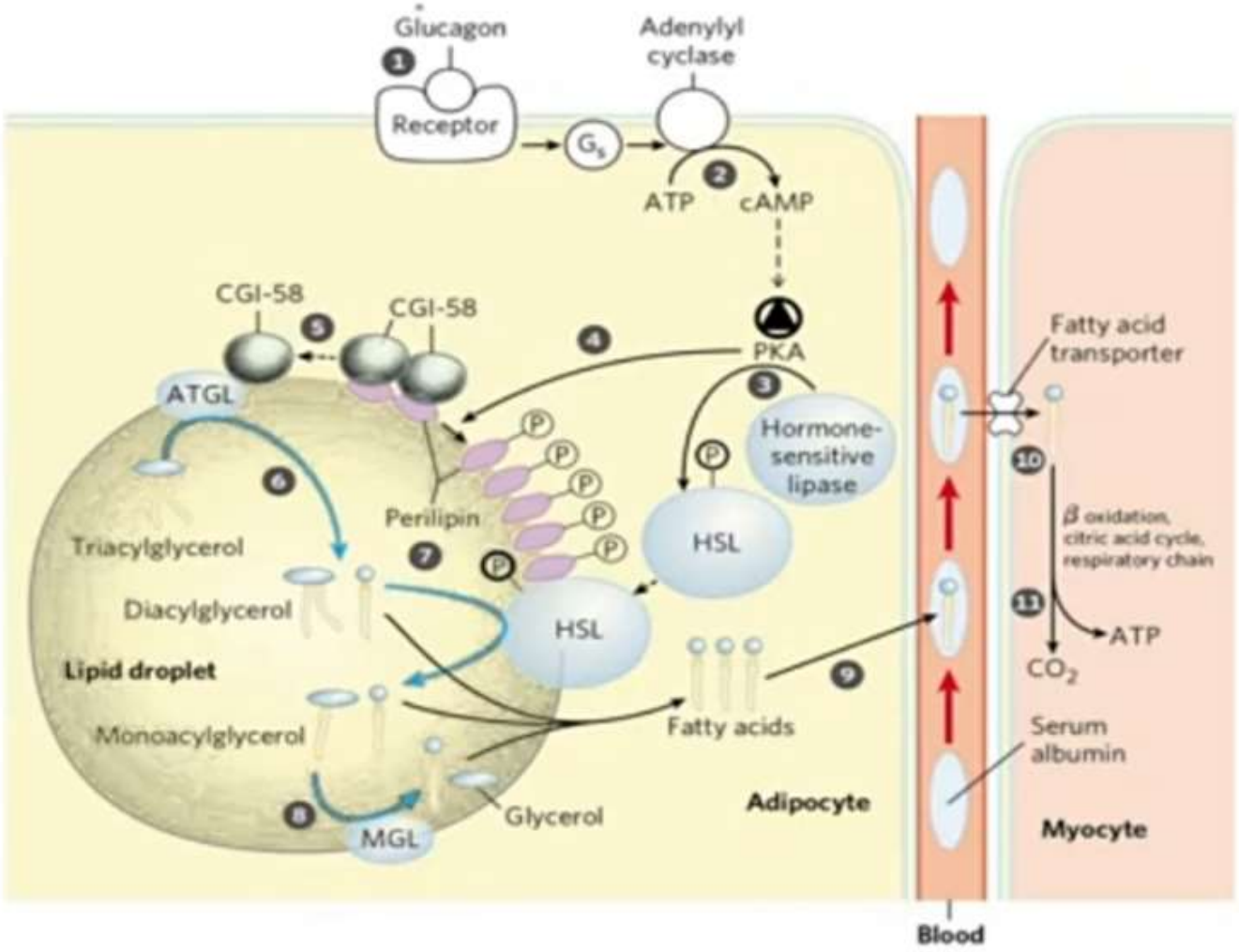
- Fatty acid oxidation is the mitochondrial aerobic process of breaking down a fatty acid into acetyl-CoA units. Fatty acids move in this pathway as CoA derivatives utilizing NAD and FAD.
- Fatty acids are activated before oxidation, utilizing ATP in the presence of CoA-SH and acyl-CoA synthetase. Long-chain acyl-CoA enters mitochondria bound to carnitine. Inside mitochondria beta oxidation of fatty acids takes place in which two carbon atoms are removed in the form of acetyl-CoA from acyl-CoA at the carboxyl terminal. The bond is broken between the second carbon/beta carbon and the third carbon/gamma carbon, hence the name beta oxidation. This process provides energy from fats.

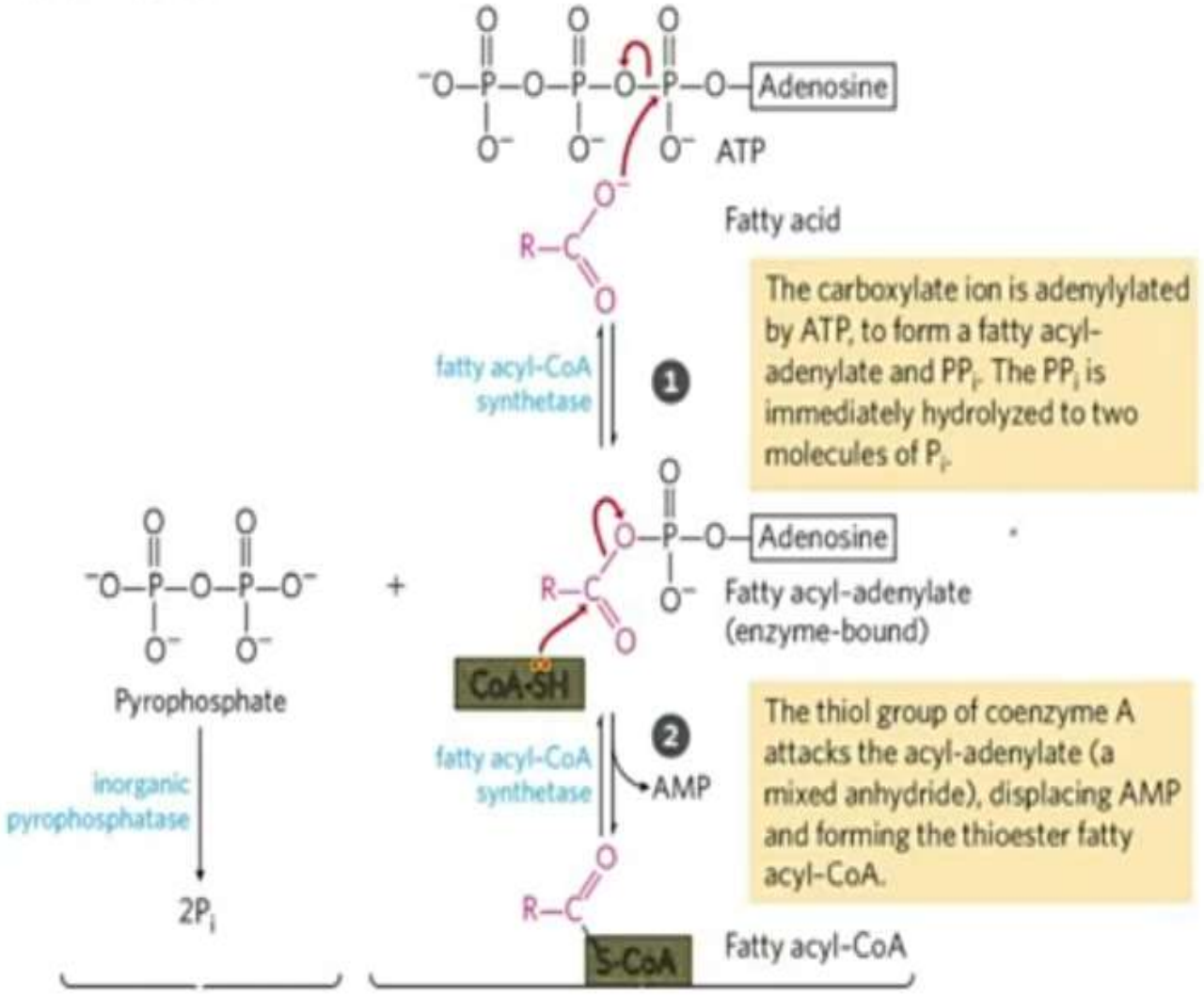
Transport

- The fatty acids released in the digestion of triglycerides and other lipids are broken down in a series of sequential reactions accompanied by the gradual release of usable energy. Some of these reactions are oxidative and require nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD).
- The enzymes that participate in fatty acid catabolism are located in the **mitochondria**, along with the enzymes of the citric acid cycle, the electron transport chain, and oxidative phosphorylation. This localization of enzymes in the mitochondria is of the utmost importance because it facilitates efficient utilization of energy stored in fatty acids and other molecules.
- Fatty acid oxidation is initiated on the outer mitochondrial membrane. There the fatty acids, which like carbohydrates are relatively inert, must first be activated by conversion to an energy-rich fatty acid derivative of coenzyme A called *fatty acyl-coenzyme A* (CoA). The activation is catalyzed by *acyl-CoA synthetase*.

- For each molecule of fatty acid activated, one molecule of coenzyme A and one molecule of adenosine triphosphate (ATP) are used, equaling a net utilization of the two high-energy bonds in one ATP molecule (which is therefore converted to adenosine monophosphate [AMP] rather than adenosine diphosphate [ADP])

- The fatty acyl-CoA diffuses to the inner mitochondrial membrane, where it combines with a carrier molecule known as **carnitine** in a reaction catalyzed by *carnitine acyltransferase*. The acyl-carnitine derivative is transported into the mitochondrial matrix and converted back to the fatty acyl-CoA.
- Carnitine is an amino acid derivative synthesized from methionine and lysine.


Glucagon
1
Receptor
G_s
Adenylyl cyclase
2
ATP
cAMP
PKA
3
4
CGI-58
5
CGI-58
ATGL
6
Perilipin
Hormone-sensitive lipase
HSL
7
HSL
Triacylglycerol
Diacylglycerol
Lipid droplet
Monoacylglycerol
8
MGL
Glycerol
Fatty acids
9
Adipocyte
Fatty acid transporter
10
β oxidation, citric acid cycle, respiratory chain
11
ATP
CO_2
Serum albumin
Myocyte
Blood


Adenosine
ATP
Fatty acid
fatty acyl-CoA synthetase
1
The carboxylate ion is adenylylated by ATP, to form a fatty acyl-adenylate and PPi. The PPi is immediately hydrolyzed to two molecules of Pi.
Adenosine
Fatty acyl-adenylate (enzyme-bound)
+
Pyrophosphate
CoA-SH
fatty acyl-CoA synthetase
2
AMP
The thiol group of coenzyme A attacks the acyl-adenylate (a mixed anhydride), displacing AMP and forming the thioester fatty acyl-CoA.
inorganic pyrophosphatase
2Pi
S-CoA
Fatty acyl-CoA

- Fatty Acid Oxidation
- To be oxidised in mitochondria, fatty acids need to reach the mitochondrial matrix. Fatty acids with chain lengths of 12 carbons or fewer enter mitochondria without the help of membrane transporters.
- However, most fatty acids are 14 or more carbons in length and require membrane transporters to enter the mitochondrial matrix.
- The transport is initiated by one of the ACSLs located in the outer mitochondrial membrane. Formed acyl-CoAs can be either transported into the mitochondrial matrix and oxidised to produce ATP, or used by mitochondrial glycerol-3-phosphate acyltransferase to synthesise lysophoshatidic acid, the first step in the glycerol-3-phosphate pathway
- Acyl-CoA destined for mitochondrial oxidation becomes the substrate for a membrane-bound CPT-I, which catalyses the production of acyl-carnitine from acyl-CoA. Acyl-carnitine is transported across the outer mitochondrial membrane and reaches the inner mitochondrial membrane, where it is received by carnitine:acylcarnitine translocase, a protein that transports both carnitine and acyl-carnitine to the cytosol and to the mitochondrial matrix, respectively.

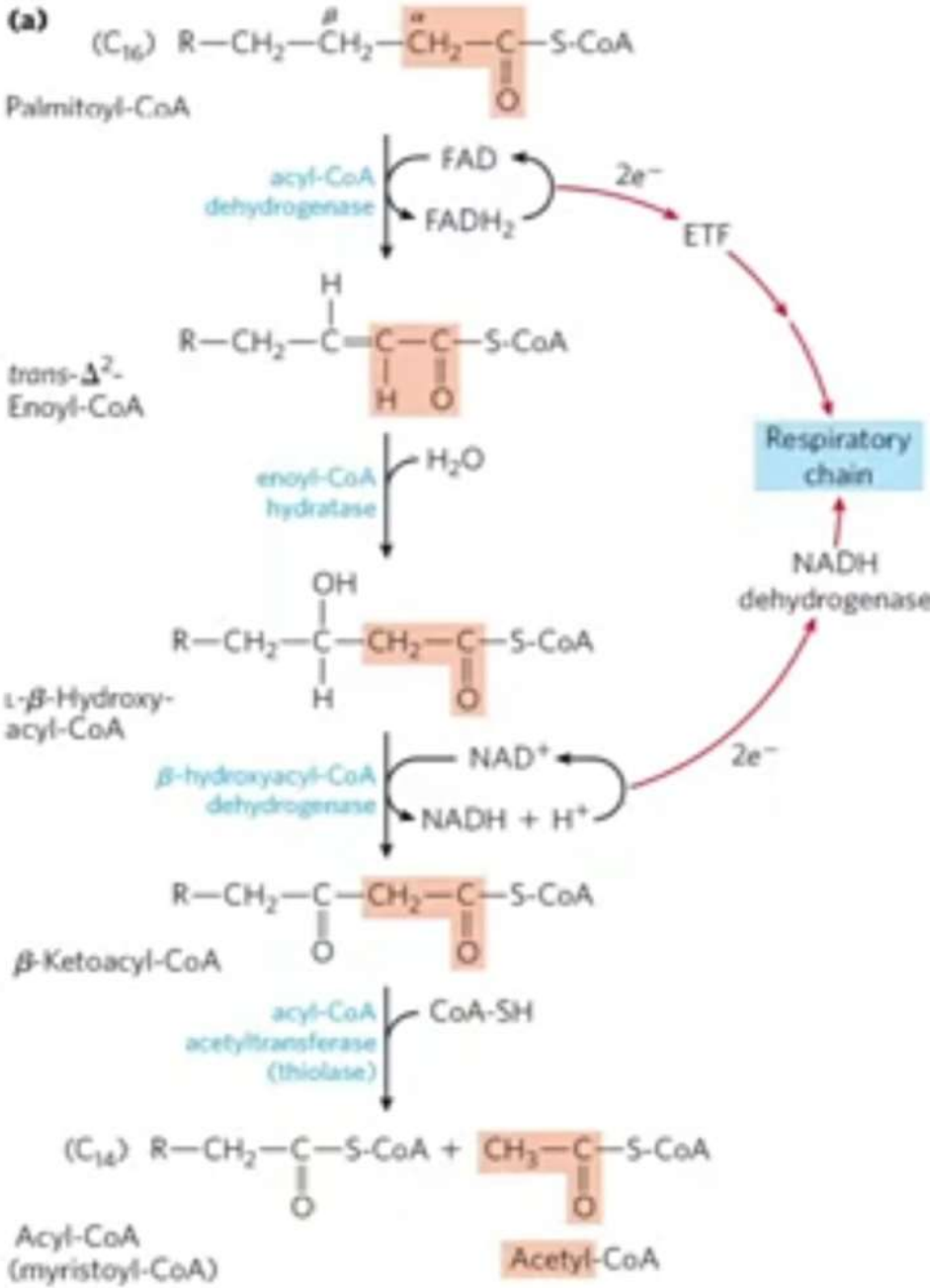
- Once the acyl-carnitine is in the mitochondrial matrix, CPT-II catalyses the reversible transfer between carnitine and CoA to regenerate acyl-CoA that then enters the β-oxidation pathway. It is important to note that the carnitine-mediated entry process is the rate-limiting step for oxidation of fatty acids in mitochondria. Mitochondrial fatty acid oxidation occurs in three main steps.
- The first step is β-oxidation, where fatty acids undergo oxidative removal of successive two-carbon units as acetyl-CoA, starting at carbon atom 3, the β-carbon of the fatty acyl chain. The process involves the action of acyl-CoA dehydrogenase, enoyl-CoA hydratase, hydroxyacyl-CoA dehydrogenase and ketoacyl-CoA thiolase.
- A 16-carbon fatty acid (palmitic acid) undergoes seven passes through this oxidative sequence, losing two carbons as acetyl-CoA on each cycle to yield total of eight molecules of acetyl-CoA.
- The second step, which also occurs in the mitochondrial matrix, involves the oxidation of acetyl-CoA to CO_2 in the citric acid cycle. In the third step, flavin adenine dinucleotide and nicotinamide adenine dinucleotide (NADH) produced during β-oxidation are used by the electron transportchain to generate ATP. Electrons derived from the first two steps pass to O_2 via the mitochondrial respiratory chain, providing the energy for ATP synthesis by oxidative phosphorylation.

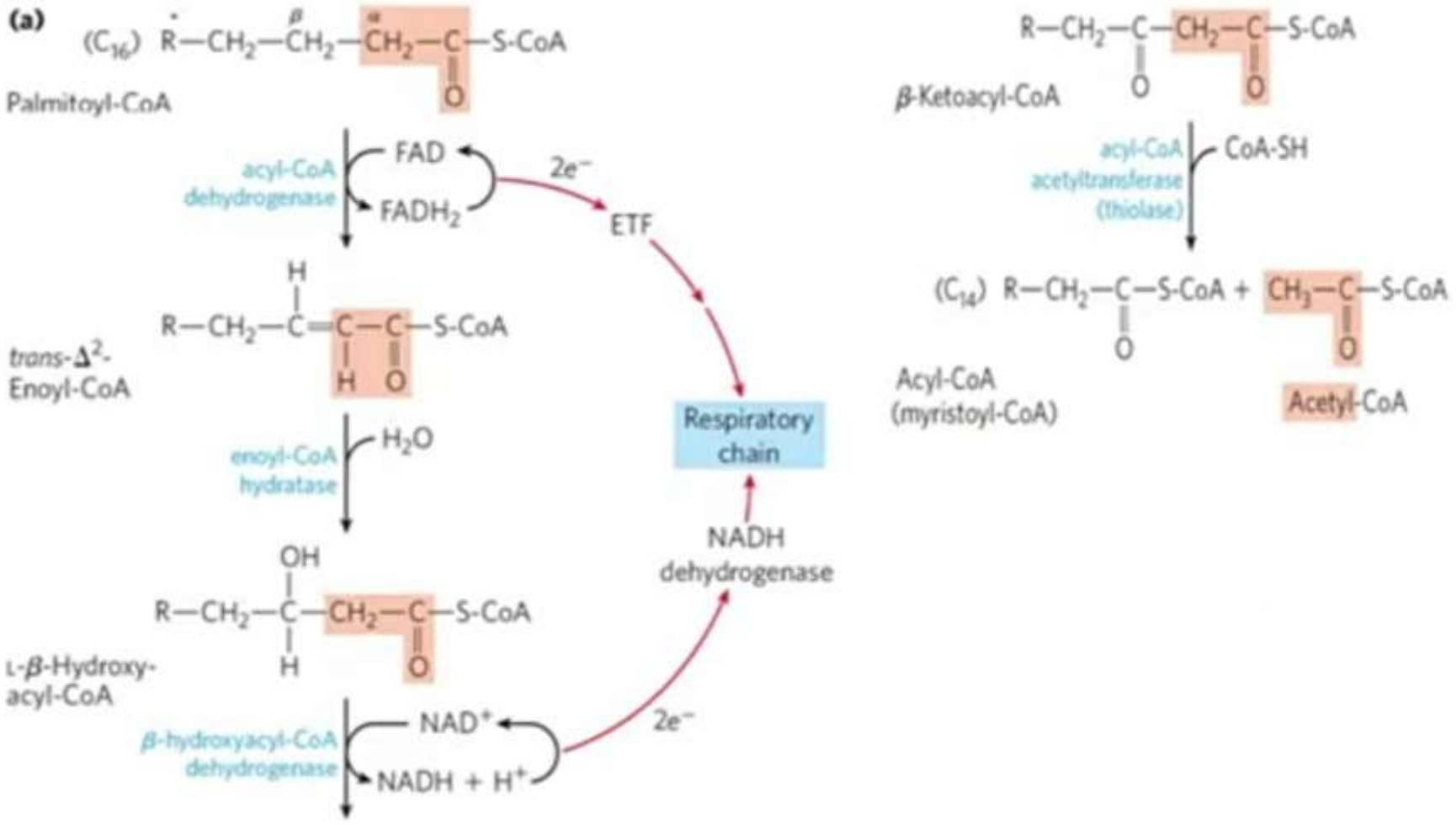
- The net yield for the complete oxidation of a palmitic acid molecule is 129 ATP molecules. Some naturally occurring fatty acids contain an odd number of carbon atoms.
- hese fatty acids are also oxidised by β-oxidation through removing two carbons as acetyl-CoA in each round of the oxidative process. However, the final products of the thiolytic cleavage of fatty acid with an odd number of carbon atoms are acetyl-CoA and propionyl-CoA (a three carbon molecule).
- Propionyl-CoA metabolism requires three more catabolic steps to be completely metabolised into succinyl-CoA that can be finally metabolised to oxaloacetate, a substrate for gluconeogenesis

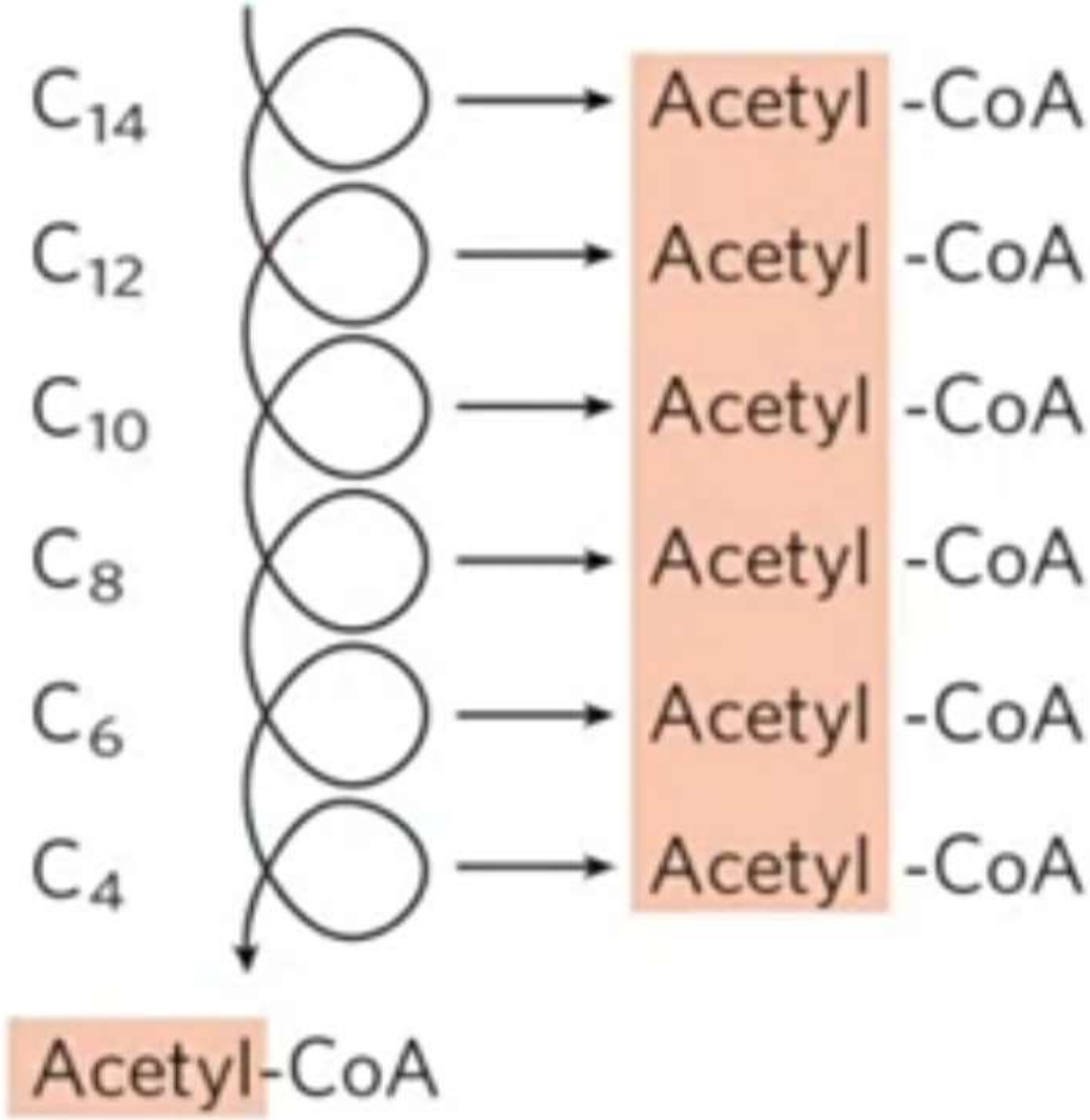
Oxidation of Fatty acid

- The first step in the catabolism of fatty acids is the formation of an alkene in an oxidation reaction catalyzed by *acyl-CoA dehydrogenase*. In this reaction, the coenzyme FAD accepts two hydrogen atoms from the acyl-CoA, one from the α-carbon and one from the β-carbon, forming reduced flavin adenine dinucleotide ($FADH_2$).
- The $FADH_2$ is reoxidized back to FAD via the electron transport chain that supplies energy to form 1.5–2 molecules of ATP.
- Next, the *trans*-alkene is hydrated to form a secondary alcohol in a reaction catalyzed by *enoyl-CoA hydratase*. The enzyme forms only the L-isomer.
- The secondary alcohol is then oxidized to a ketone by *β-hydroxyacyl-CoA dehydrogenase*, with NAD^+ acting as the oxidizing agent. The reoxidation of each molecule of NADH to NAD^+ by the electron transport chain furnishes 2.5–3 molecules of ATP.
- The final reaction is cleavage of the β-ketoacyl-CoA by a molecule of coenzyme A. The products are acetyl-CoA and a fatty acyl-CoA that has been shortened by two carbon atoms. The reaction is catalyzed by *thiolase*.

Oxidation of Fatty Acids




(a)
(C16) R—CH2—CH2—CH2—C—S-CoA
Palmitoyl-CoA
acyl-CoA dehydrogenase
FAD
FADH2
2e−
ETF
trans-Δ2-Enoyl-CoA
enoyl-CoA hydratase
H2O
L-β-Hydroxy-acyl-CoA
β-hydroxyacyl-CoA dehydrogenase
NAD+
NADH + H+
2e−
NADH dehydrogenase
Respiratory chain
β-Ketoacyl-CoA
acyl-CoA acetyltransferase (thiolase)
CoA-SH
(C14) R—CH2—C—S-CoA + CH3—C—S-CoA
Acyl-CoA (myristoyl-CoA)
Acetyl-CoA


C14
C12
C10
C8
C6
C4
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA
Acetyl-CoA

The shortened fatty acyl-CoA is then degraded by repetitions of these four steps, each time releasing a molecule of acetyl-CoA. The overall equation for the β-oxidation of palmitoyl-CoA (16 carbon atoms) is as follows:

$$\text{Palmitoyl-CoA} + 7\text{CoA} + 7\text{FAD} + 7\text{NAD}^+ + 7\text{H}_2\text{O}$$
$$\rightarrow\ 8\ \text{acetyl-CoA} + 7\text{FADH}_2 + 7\text{NADH} + 7\text{H}^+$$

I) Palmitoyl-CoA + 7CoA + $7O_2$ + $28P_i$ + 28ADP →

8Acetyl-CoA + 28 ATP + $7H_2O$

II) Acetyl-CoA oxidized in the TCA cycle

1Acetyl-CoA → 10ATP

8Acetyl-CoA → 80ATP

8Acetyl-CoA + $16O_2$ + $80P_i$ + 80ADP →

8 CoA + 80ATP + $16H_2O$ + $16CO_2$

Combine I and II

Palmitoyl-CoA + $23O_2$ + $108P_i$ + 108ADP →

CoA + 108ATP + $16CO_2$ + $23H_2O$

ATP Yield from Fatty Acid Oxidation. Comparison with Glucose Oxidation.

- The amount of ATP obtained from fatty acid oxidation depends on the size of the fatty acid being oxidized. For our purposes here. we'll study palmitic acid, a saturated fatty acid with 16 carbon atoms, as a typical fatty acid in the human diet. Calculating its energy yield provides a model for determining the ATP yield of all other fatty acids.
- The breakdown by an organism of 1 mol of palmitic acid requires 1 mol of ATP (for activation) and forms 8 mol of acetyl-CoA. Recall from the metabolism of carbohydrates that each mole of acetyl-CoA metabolized by the citric acid cycle yields 12 mol of ATP. The complete degradation of 1 mol of palmitic acid requires the β-oxidation reactions to be repeated seven times. Thus, 7 mol of NADH and 7 mol of $FADH_2$ are produced. Reoxidation of these compounds through respiration yields 3 and 2 mol of ATP, respectively. The energy calculations can be summarized as follows:
- 1 mol of ATP is split to AMP and $2P_i$ −2 ATP
- 8 mol of acetyl-CoA formed (8 × 12 ATP) 96 ATP
- 7 mol of $FADH_2$ formed (7 × 2) 14 ATP
- 7 mol of NADH formed (7 × 3) 21 ATP
- **Total** 129 ATP
- The number of times β-oxidation is repeated for a fatty acid containing n carbon atoms is n/2 – 1 because the final turn yields two acetyl-CoA molecules.
- The combustion of 1 mol of palmitic acid releases a considerable amount of energy:
- $C16H32O2+23O2 \rightarrow 16CO2+16H2O+2,340kcal$

- The percentage of this energy that is conserved by the cell in the form of ATP is as follows:
- Energy conserved /total energy available×100=(129ATP) (7.4kcal/ATP)/2,340kcal×100=41%

Energy conserved total energy available×100=(129ATP)(7.4kcal/ATP)/2,340kcal×100=41%

- In terms of moles of reactant, the efficiency of fatty acid metabolism is comparable to that of carbohydrate metabolism, which we calculated to be 42%. However, in terms of grams, there is a big difference between the energy that can be generated per gram of glucose and per gram of fatty acid.
- In the carbohydrate metabolism module, we determine that the oxidation of 1 mol of glucose produces 38 ATP moles, that is, 38 x 7.4 kcal /mol ATP = 281.2 kcal. That is the amount of energy produced by 1 mol, or 180 g of glucose. In other words, 1 gram of glucose produces 1.56 kcal of energy (1.56/g glucose). For a fatty acid, such as palmitic acid, we are able to produce 129 ATP molees per mol of palmitic acid, that is, 129 x 7.4 kcal/mol ATP = 954.6 kal. One mole of palmitic acid equals 256 grams. Therefore, the complete oxidation of palmitic acid produces 3.72 kcal/g of palmitic acid, which is **more than twice the amount of energy obtained per mole of glucose**. The fact that carbons atoms in fatty acids are more reduced than the carbon atoms in glucose explains the difference in the amount of energy produced by their oxidation.

Interesting fact: te oxidation of fatty acids also produces large quantities of water. This water, which sustains migratory birds and animals (such as the camel) for long periods of time.

- Chains with an odd-number of carbons are oxidized in the same manner as even-numbered chains, but the final products are propionyl-CoA and Acetyl CoA

REFERENCES

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- Esentials of Biochemistry U.Satyanarayana,U.Chakrapani
- https://www.sciencedirect.com

HOLLIDAY MODEL

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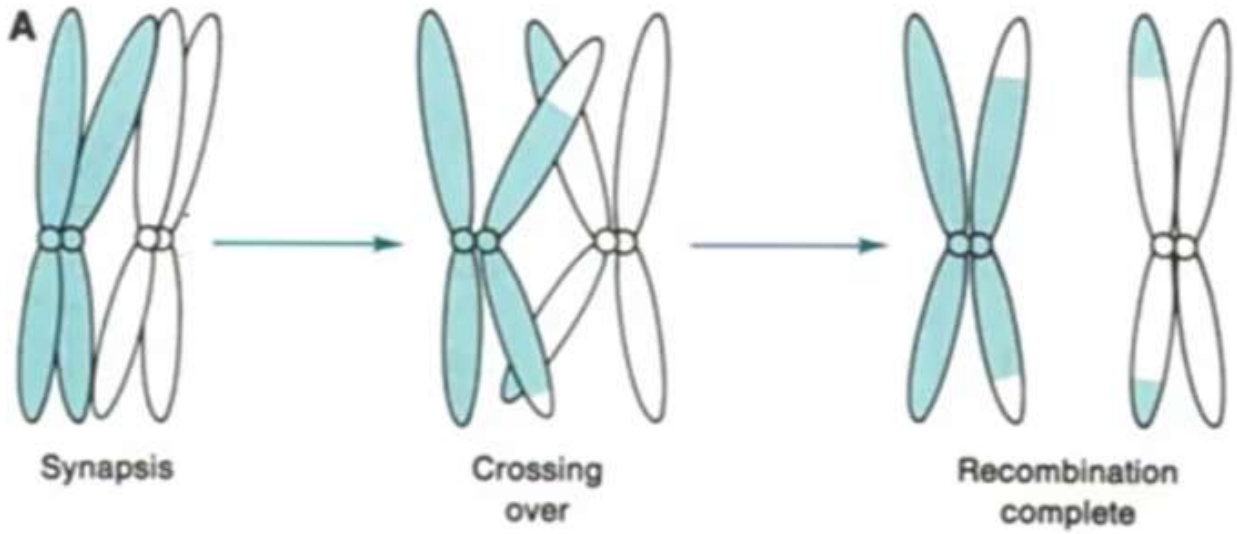
Introduction

- In 1964, Robin Holliday proposed a model that accounted for heteroduplex formation and gene conversion during recombination. Although it has been supplanted by the double-strand break model (at least for recombination in yeast and higher organisms), it is a useful place to start. It illustrates the critical steps of pairing of homologous duplexes, formation of a heteroduplex, formation of the recombination joint, branch migration and resolution.

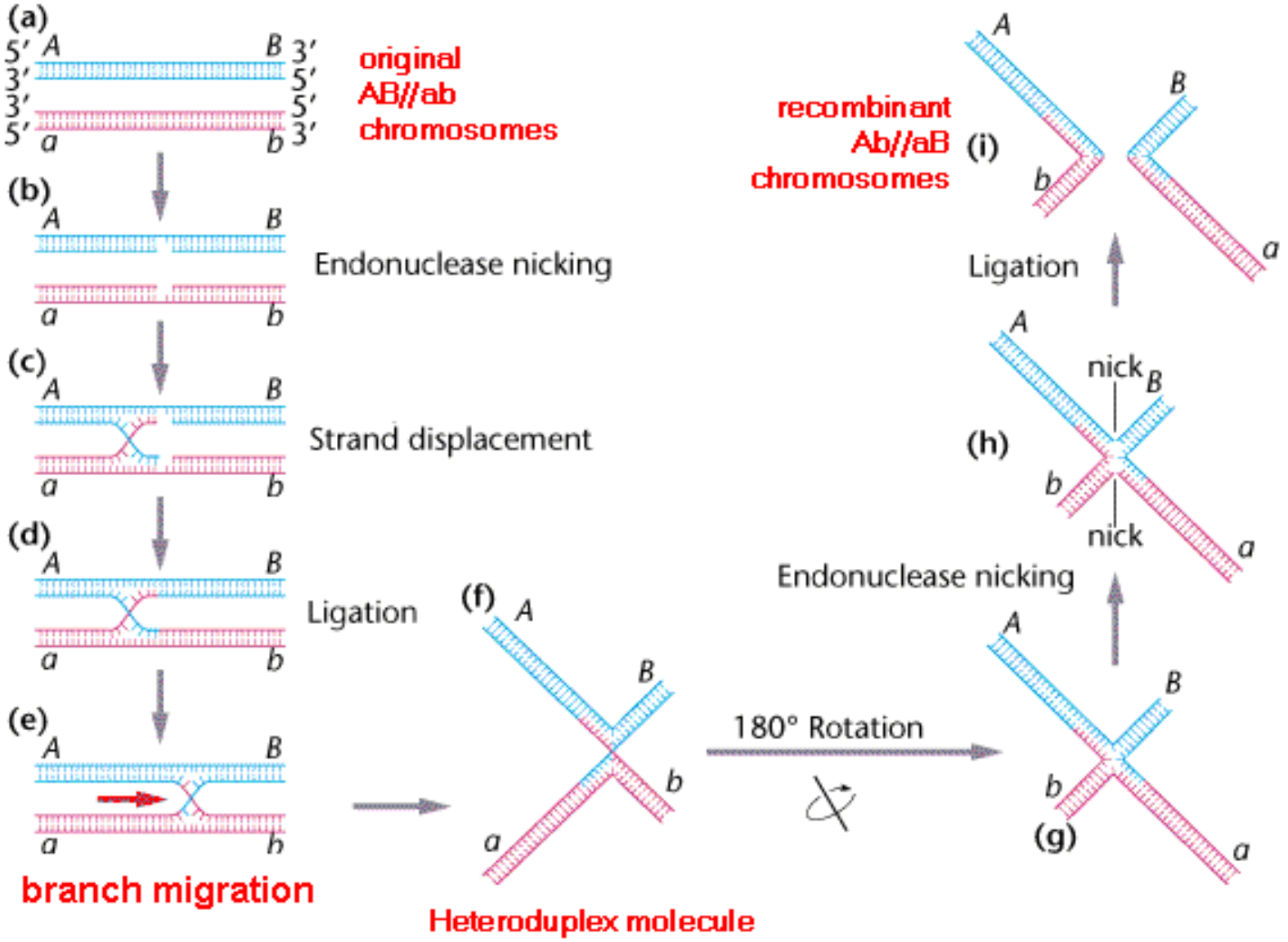
- The steps in the Holliday Model are,
- Two homologous chromosomes, each composed of duplex DNA, are **paired** with similar sequences adjacent to each other.
- An endonuclease **nicks** at corresponding regions of homologous strands of the paired duplexes. This is shown for the strands with the arrow to the right in the figure.
- The nicked ends dissociate from their complementary strands and each **single strand invades the other duplex**. This occurs in a reciprocal manner to produce a **heteroduplex region**derived from one strand from each parental duplex.
- DNA ligase **seals the nicks**. The result is a stable **joint molecule**, in which one strand of each parental duplex crosses over into the other duplex.This X-shaped joint is called a **Holliday intermediate** or **Chi structure**.
- Branch migration then expands the region of heteroduplex. The stable joint can move along the paired duplexes, feeding in more of each invading strand and extending the region of heteroduplex.
- The recombination intermediate is then **resolved** by nicking a strand in each duplex and ligation.


A
Synapsis
Crossing over
Recombination complete

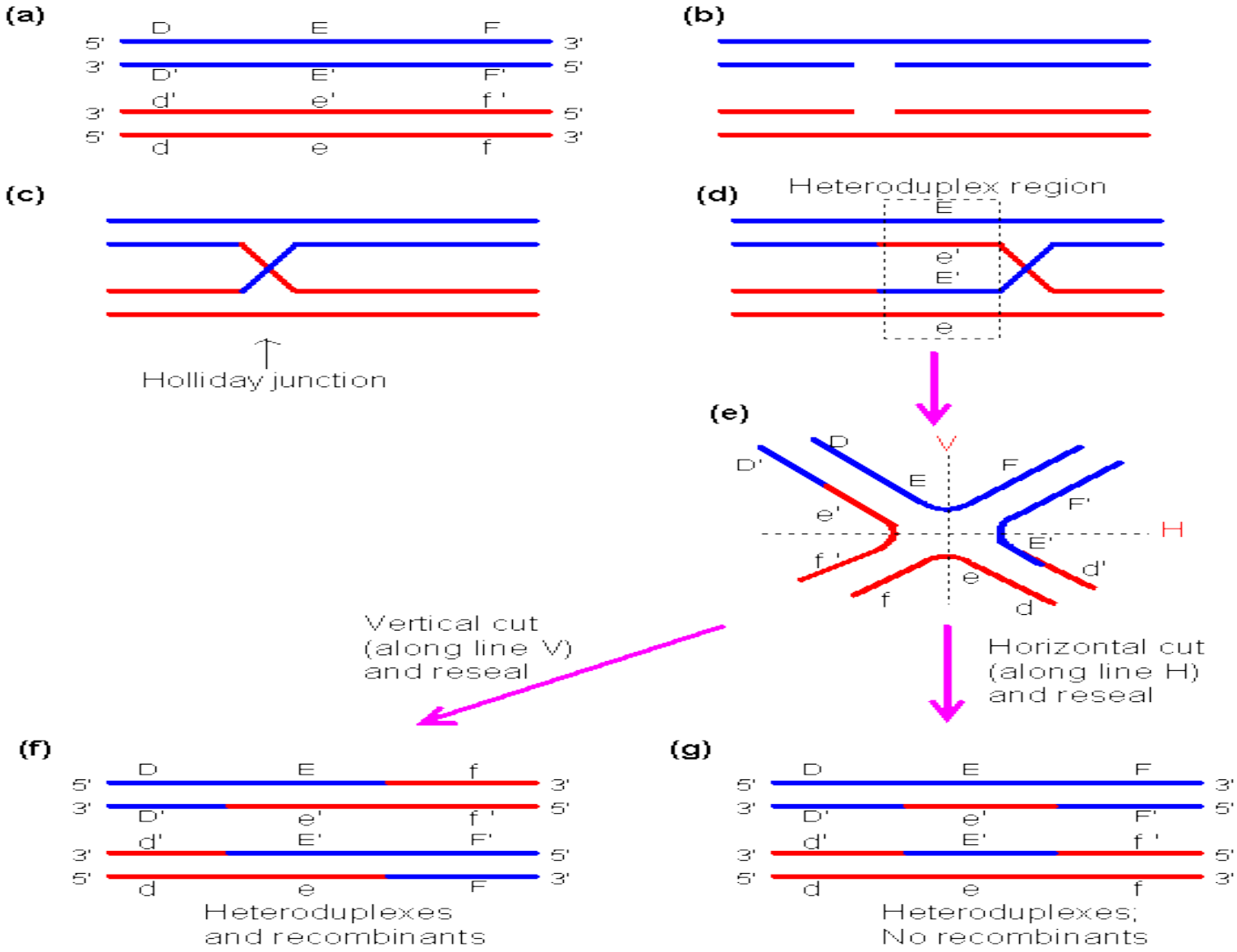
- The key features of the Holliday model are the formation of heteroduplex DNA; the creation of a cross bridge; its migration along the two heteroduplex strands, termed branch migration; the occurrence of mismatch repair; and the subsequent resolution, or splicing, of the intermediate structure to yield different structures

Mechanism of Holliday Model

- The Holliday junction is a cross-stranded structure where the two DNA duplexes intersect, and the heteroduplex is the stretch of double-stranded DNA that consists of single strands from different homologous chromosomes



Holliday-Whitehouse model of recombination (after Klug & Cummings 1997)


(a)
D
E
F
5'
3'
3'
5'
D'
E'
F'
d'
e'
f '
3'
5'
5'
3'
d
e
f
(b)
(c)
Holliday junction
(d)
Heteroduplex region
E
e'
E'
e
(e)
D
V
D'
E
F
e'
F'
H
E'
f '
d'
e
f
d
Vertical cut
(along line V)
and reseal
Horizontal cut
(along line H)
and reseal
(f)
D
E
f
5'
3'
3'
5'
D'
e'
f '
d'
E'
F'
3'
5'
5'
3'
d
e
F
Heteroduplexes
and recombinants
(g)
D
E
F
5'
3'
3'
5'
D'
e'
F'
d'
E'
f '
3'
5'
5'
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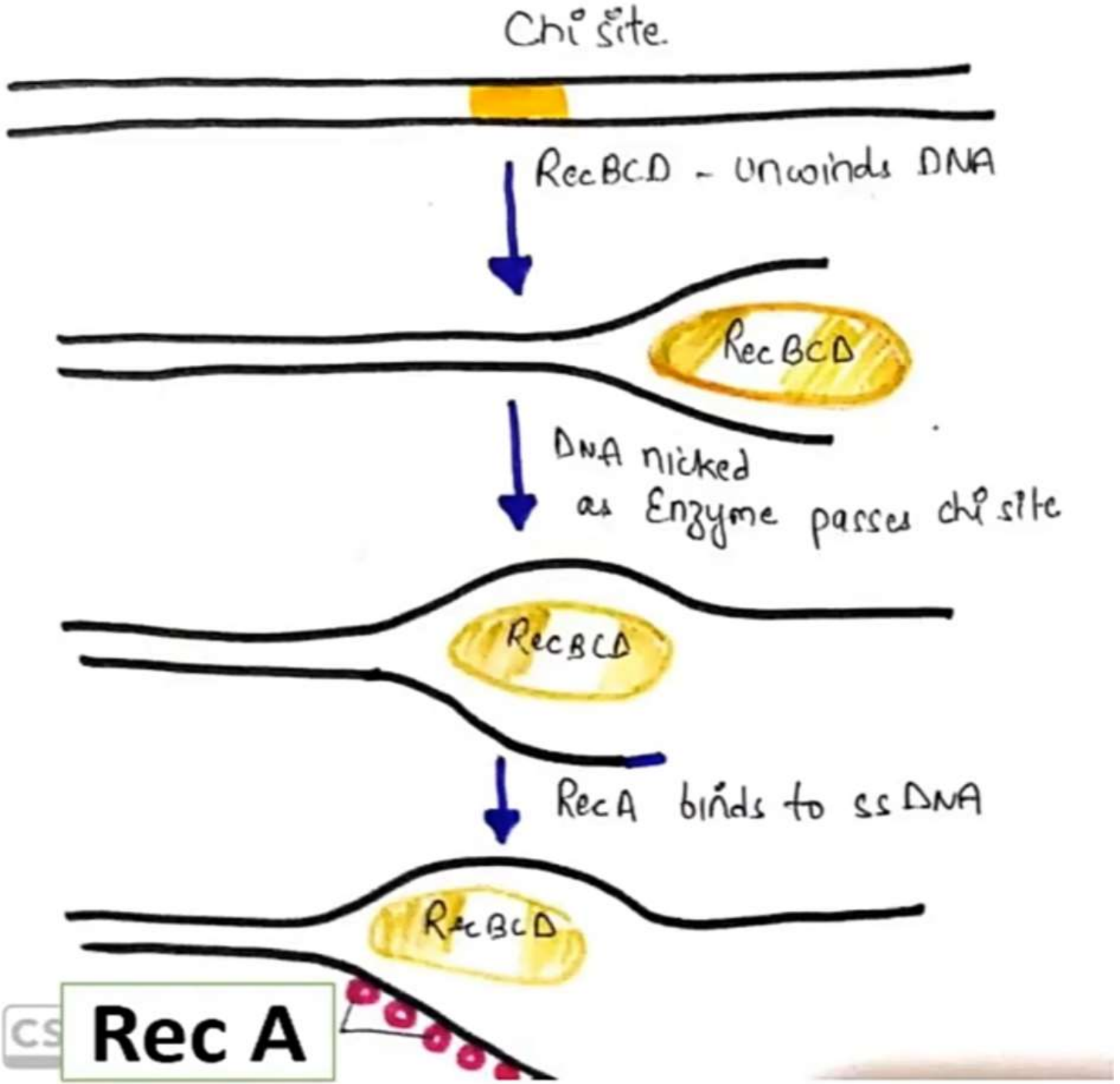
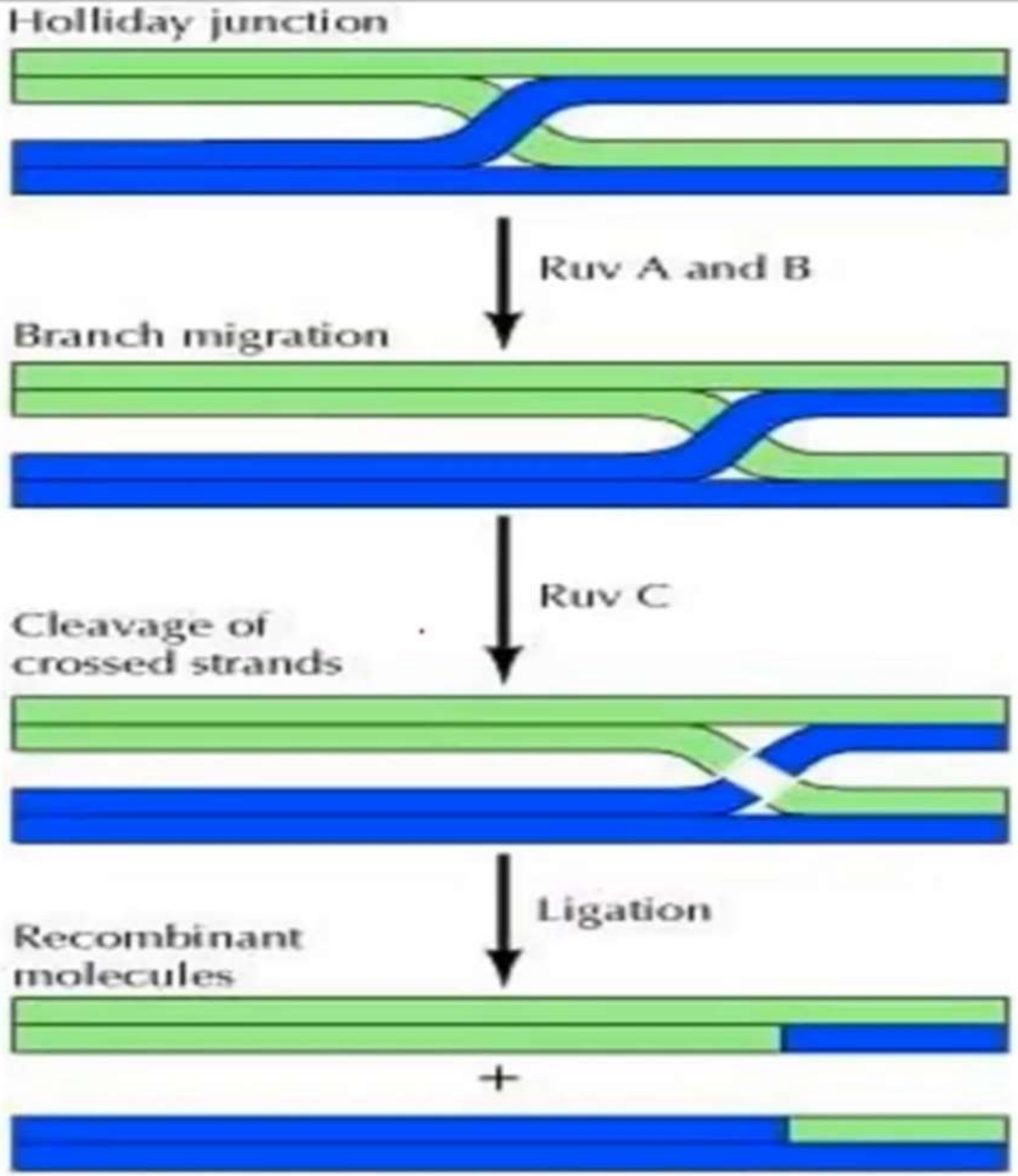
d
e
f
Heteroduplexes;
No recombinants


Chi site.
RecBCD - unwinds DNA
RecBCD
DNA nicked
as Enzyme passes chi site
RecBCD
RecA binds to ss DNA
RecBCD
Rec A


Holliday junction
Ruv A and B
Branch migration
Ruv C
Cleavage of
crossed strands
Ligation
Recombinant
molecules
+

Reference

- https://byjus.com
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- Sandy B. Primrose

Primary and secondary Structure of protein

Presented by,

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Assistant Professor

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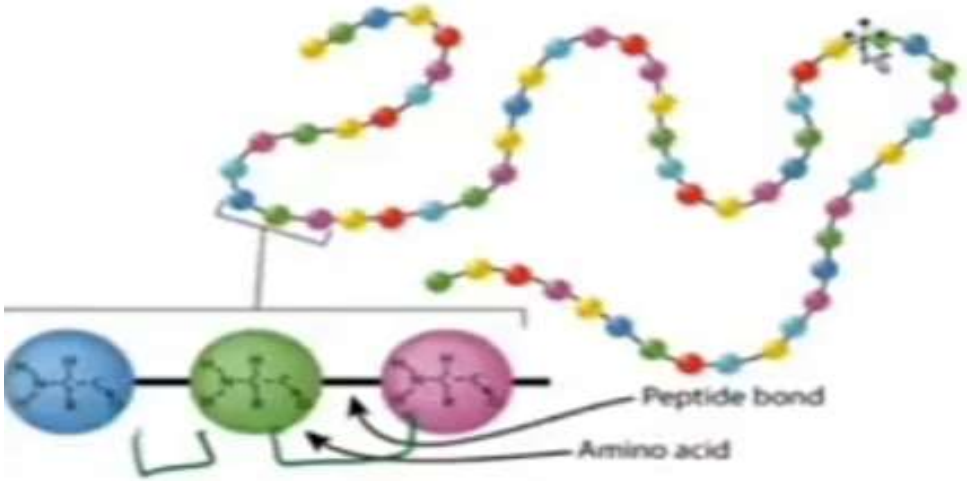
Introduction

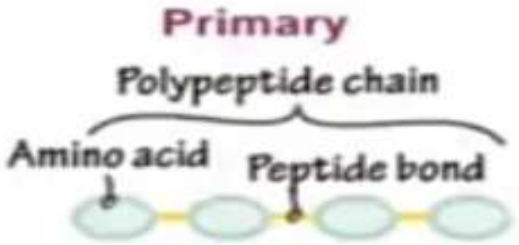
- Protein structures are made by condensation of amino acids forming peptide bonds. The sequence of amino acids in a protein is called its primary structure. The secondary structure is determined by the dihedral angles of the peptide bonds, the tertiary structure by the folding of protein chains in space. Association of folded polypeptide molecules to complex functional proteins results in quaternary structure.

Primary Structure

- Primary protein structure is when amino acids bound are together via **covalent peptide bonds** to form a **polypeptide chain**. These bonds form between the N terminal and C terminal of amino acids and are highly resistant to heat or chemicals.
- Any mutation in this amino acid sequence can affect protein folding, leading to problems with the protein’s function.

PRIMARY STRUCTURE



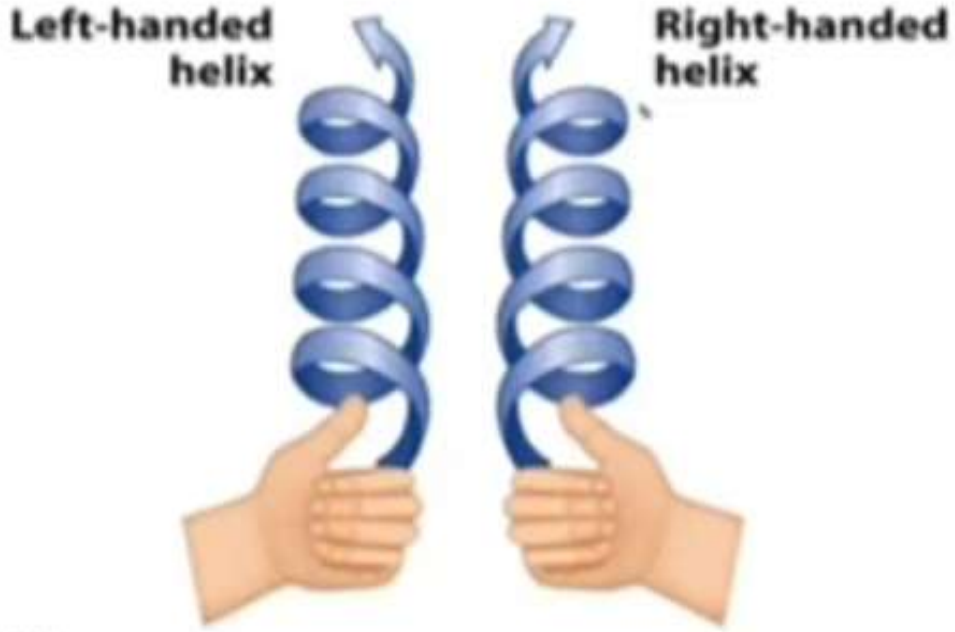


Secondary structure

- The next level of protein structure, **secondary structure**, refers to local folded structures that form within a polypeptide due to interactions between atoms of the backbone. (The backbone just refers to the polypeptide chain apart from the R groups – so all we mean here is that secondary structure does not involve R group atoms.) The most common types of secondary structures are the α helix and the β pleated sheet. Both structures are held in shape by hydrogen bonds, which form between the carbonyl O of one amino acid and the amino H of another.
- In an **α helix**, the carbonyl (C=O) of one amino acid is hydrogen bonded to the amino H (N-H) of an amino acid that is four down the chain. (E.g., the carbonyl of amino acid 1 would form a hydrogen bond to the N-H of amino acid 5.) This pattern of bonding pulls the polypeptide chain into a helical structure that resembles a curled ribbon, with each turn of the helix containing 3.6 amino acids. The R groups of the amino acids stick outward from the α helix, where they are free to interact

- In a **β pleated sheet**, two or more segments of a polypeptide chain line up next to each other, forming a sheet-like structure held together by hydrogen bonds. The hydrogen bonds form between carbonyl and amino groups of backbone, while the R groups extend above and below the plane of the sheet\[^3\]. The strands of a β pleated sheet may be **parallel**, pointing in the same direction (meaning that their N- and C-termini match up), or **antiparallel**, pointing in opposite directions (meaning that the N-terminus of one strand is positioned next to the C-terminus of the other).
- Certain amino acids are more or less likely to be found in α-helices or β pleated sheets. For instance, the amino acid proline is sometimes called a “helix breaker” because its unusual R group (which bonds to the amino group to form a ring) creates a bend in the chain and is not compatible with helix formation\[^4\]. Proline is typically found in bends, unstructured regions between secondary structures. Similarly, amino acids such as tryptophan, tyrosine, and phenylalanine, which have large ring structures in their R groups, are often found in β pleated sheets, perhaps because the β pleated sheet structure provides plenty of space for the side chains

α HELIX SECONDARY STRUCTURE



1. HYDROGEN BOND

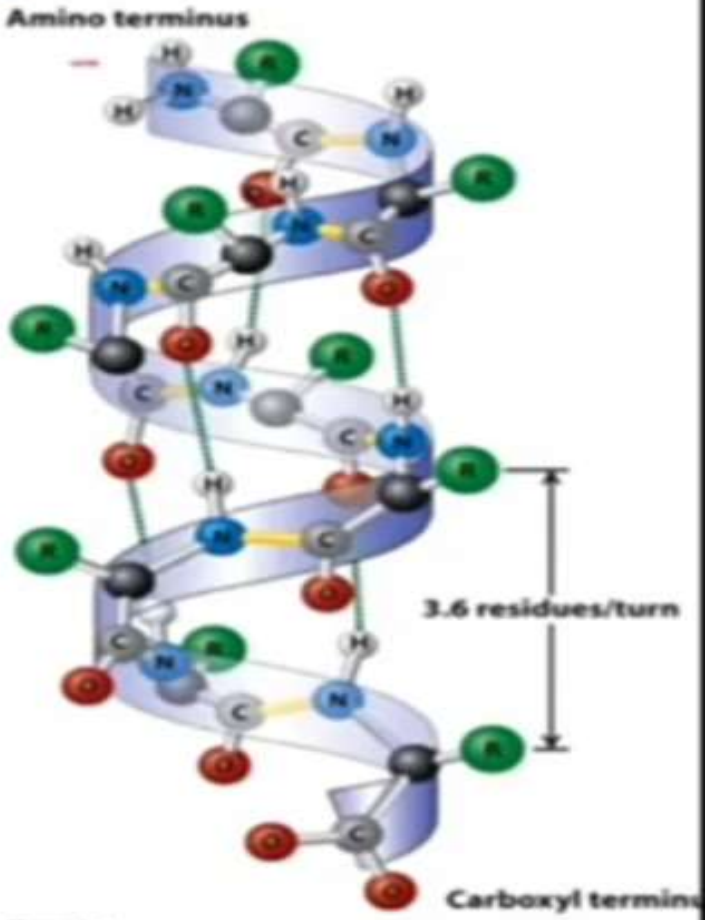
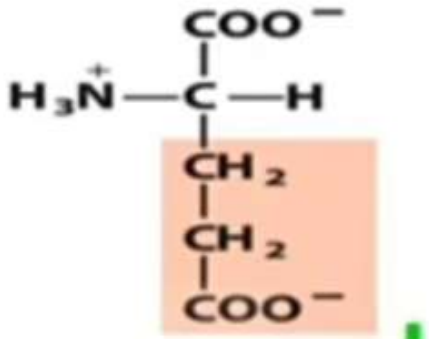


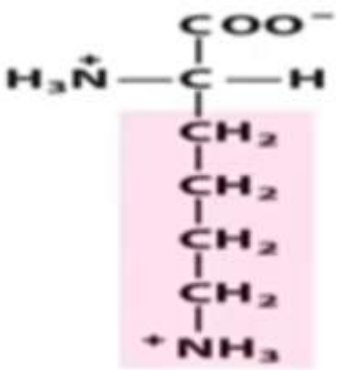
Figure 3-4
Molecular Cell Biology, Sixth Edition

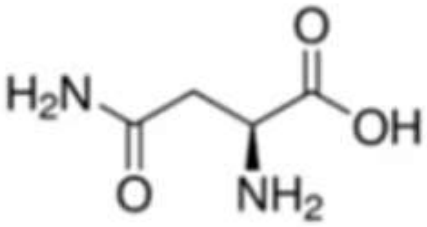
AMINO ACID AFFECTING STABILITY

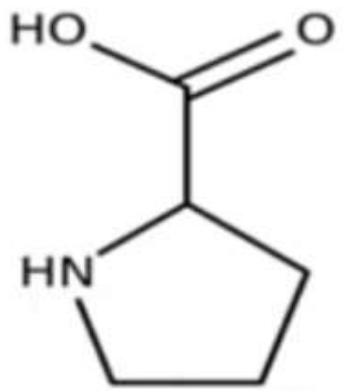


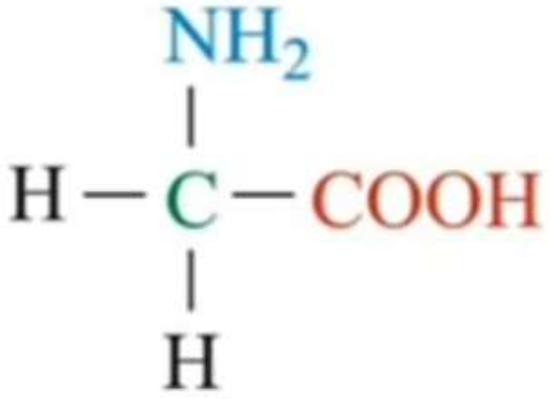
Glutamate

(Aminoglutamic acid)

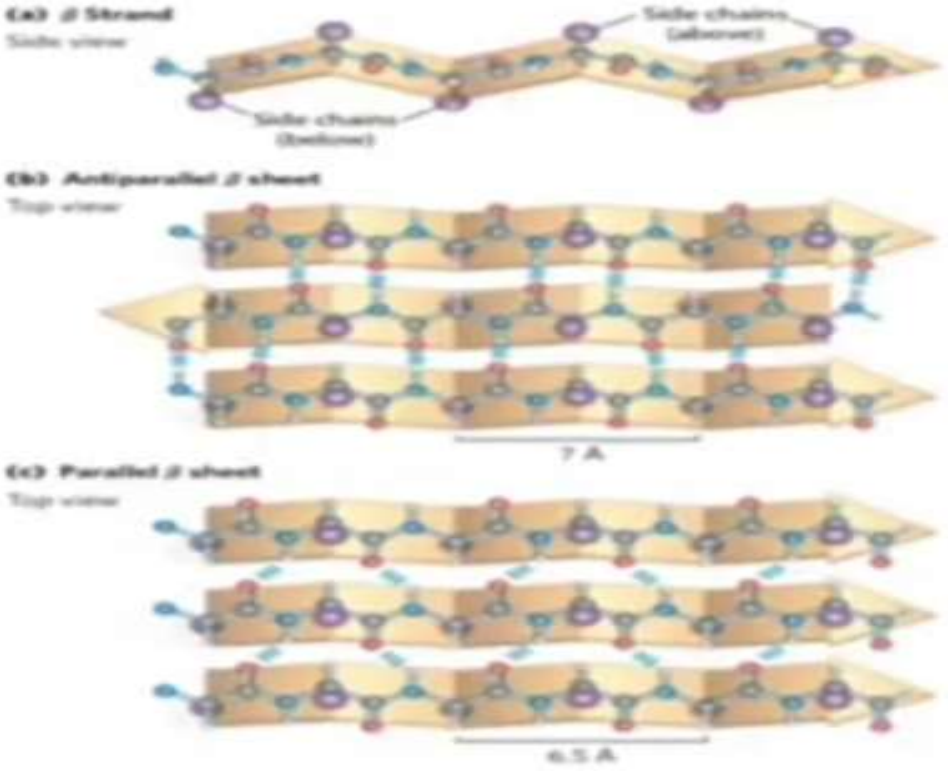




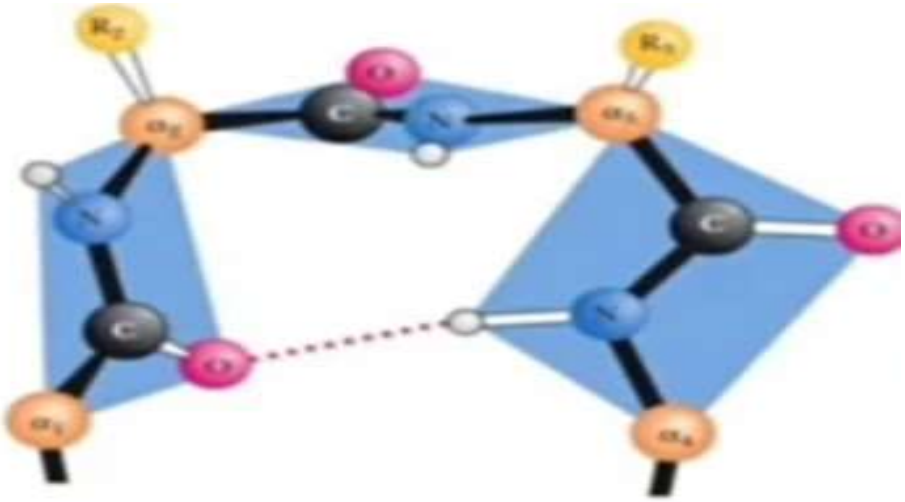

HO
O
HN

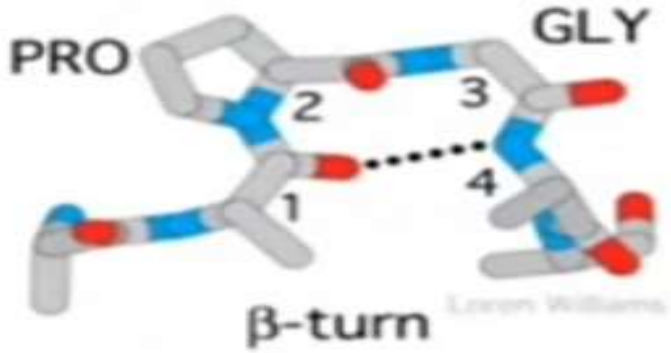

NH2
H
C
COOH
H

β Sheets


(a) β Strand
Side chains (above)
Side chains (below)
(b) Antiparallel β sheet
7 Å
(c) Parallel β sheet
6.5 Å

β Turns

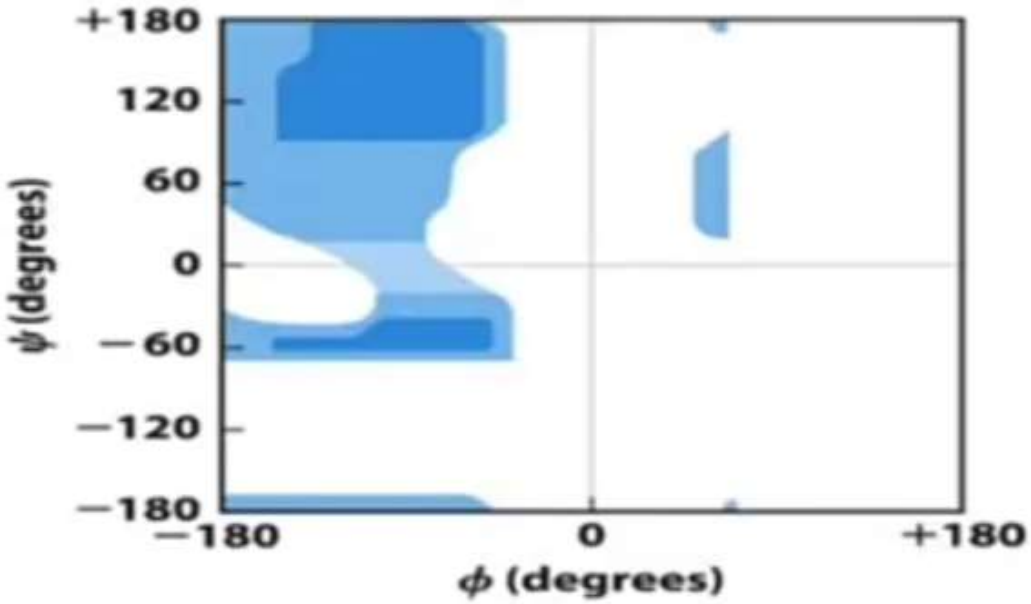


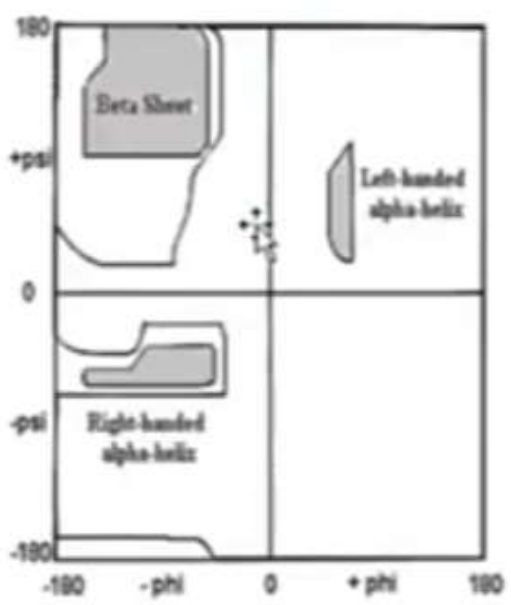


Ramchandran plot

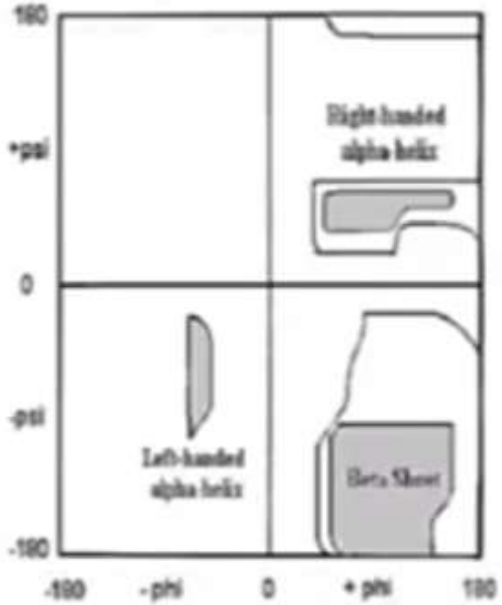
- **Ramachandran Plot** is a graphical representation of the dihedral angles (ϕ and ψ) of amino acid residues in protein structures. Regions in the plot indicate whether a protein structure is energetically favorable or not and help in checking and improving the accuracy and quality of three-dimensional representations of proteins. It is a valuable tool for understanding the structural accuracy of protein conformations.
- A **Ramachandran plot**, also known as a Ramachandran diagram or a Rama plot in the field of biochemistry, was originally developed by **Viswanathan Sasisekharan** (born in 1993), **C. Ramakrishnan** and **Gopalasamudram Narayana Ramachandran.** Ramachandran plot is the plot of angles called psi (ψ) and phi (φ) of the residues (which are commonly known as amino acids) present in a peptide. **It** assess the stereochemical quality of protein structures by plotting the phi (ϕ) and psi (ψ) dihedral angles of amino acid residues. As the partial-double-bond keeps the peptide bond planar, the ω angle at that particular peptide bond is always 180 degrees (180°).

RAMACHANDRAN PLOT





L-chiral



D-Chiral

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Slow viruses

Presented by

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Vivekanand College (Empowered Autonomous)

Introduction

- The term "slow virus disease" applied to group of infections in animals & humans characterized by a very
- - Long incubation period.
- - Slow but relentless course
- - Terminating fatally
- Concept was proposed by Singurdsson- a veterinary pathologist

Characteristics

- Incubation period ranging from months to years
- Course of illness lasting from months to years with remission & exacerbations
- Predilection for involvement of CNS
- Absence of immune response
- OR immune response that may actually contribute to pathogenesis
- Genetic predisposition
- Invariable fatal termination

Classification

Group A-

- Slowly progressive infections of sheep
  - *Visna*
  - *Maedi*
- Caused by serologically related non-oncogenic retroviruses called as lentiviruses
  Another e.g.- HIV

Group B-

- Comprising Prion disease of CNS, scrapie, mink encephalopathy, kuru, CJD.
- Are collectively known as subacute spongioform viral encephalopathy

- Group C-
  - Consisting two unrelated CNS diseases of human beings
    - *Subacute sclerosing panencephalitis(SSPE) and*
    - *Progressive multifocal encephalopathy(PML)*

Disease	Agent	Hosts	Incubation period	Nature of disease
Disease of humans				
Subacute sclerosing panencephalitis	Measles virus variant	Humans	2-20 years	Chronic sclerosing panencephalitis
Progressive multifocal leukoencephalopathy	Polyomavirus JCV	Humans	Years	Central nervous system demyelination
Creutzfeldt – Jakob disease	Prion	Humans, chimpanzees, monkeys	Months to years	Spongiform encephalopathy
Kuru	Prion	Humans, chimpanzees, monkeys	Months to years	Spongiform encephalopathy

Disease	Agent	Hosts	Incubation period	Nature of disease
Disease of animals				
Visna	Retrovirus	Sheep	Months to years	Central nervous system demyelination
Scrapie	Prion	Sheep, goats, mice	Months to years	Spongiform encephalopathy
Bovine spongiform encephalopathy	Prion	Cattle	Months to years	Spongiform encephalopathy
Transmissible mink encephalopathy	Prion	Mink, other animals	Months	Spongiform encephalopathy
Chronic wasting disease	Prion	Mule deer, elk	Months to years	Spongiform encephalopathy

Group A diseases

- **Visna**
  - Demyelinating disease of sheep
  - Characterised by paresis, paralysis & death
  - It was eradicated by slaughtering of all infected animals in 1957
- **Maedi**
  - It is slowly progressive haemorrhagic pneumonia of sheep

Group B Diseases(Prion diseases)

- **Infections in animals** -
  - Scrapie
  - Mink encephalopathy
  - Bovine spongioform encephalopathy (mad cow disease)
- **Infections in humans**
  - Creutzfeldt-Jakob disease (CJD)
  - Kuru
  - Fatal familial insomnia

Pathology

- Infective agent is proteinaceous in nature(called as proteinaceous infectious particle)
- Devoid of DNA & RNA, known as Prions
- It is usually resistant to physical & chemical agents
- Can be transmitted to animals by oral or parentral challenge
- PrP → PrPSC
- Stanley B. Prusiner gave the name Prion

Prions

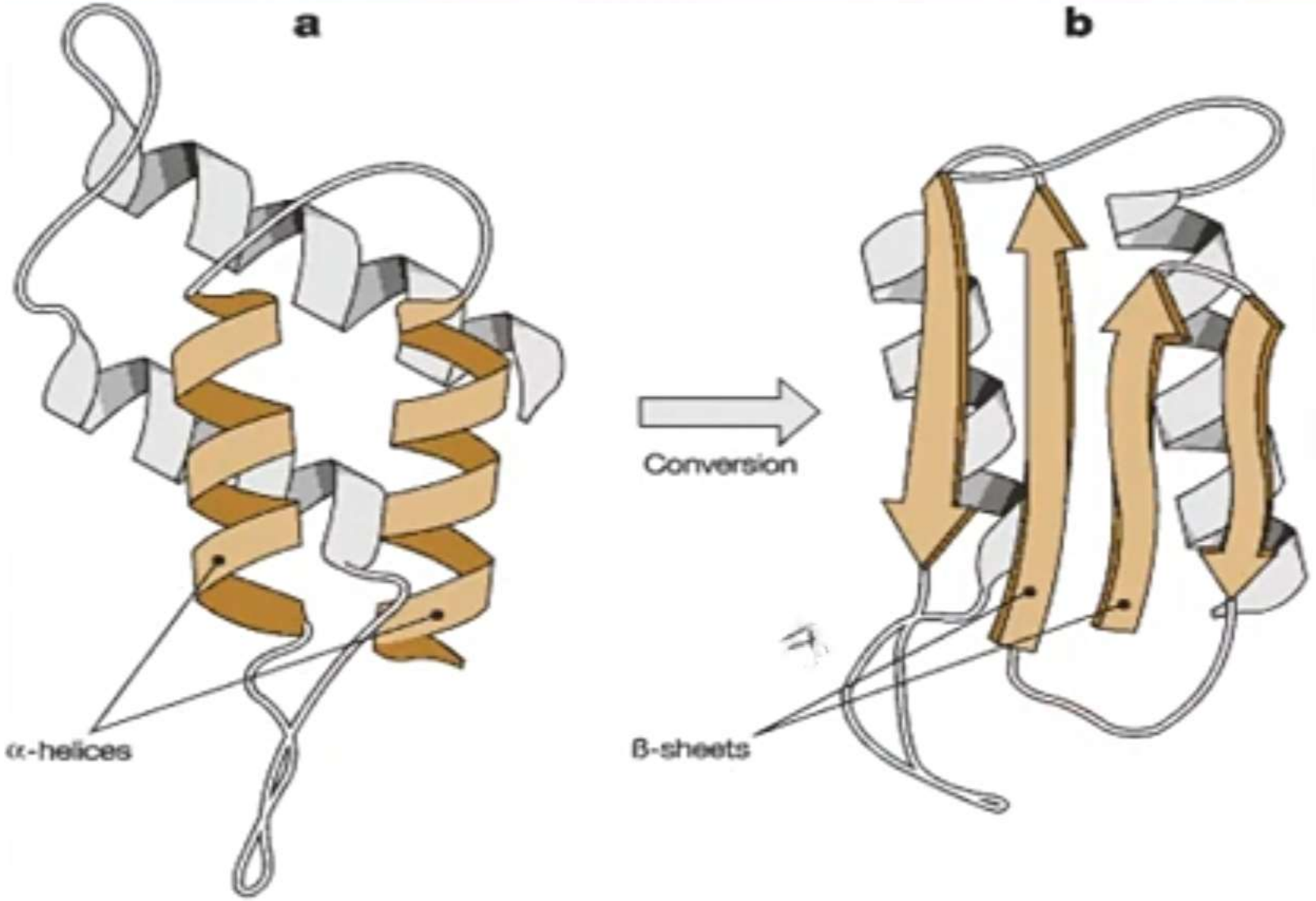
- All known mammalian prion diseases are caused by the so-called prion protein, PrP. The endogenous, properly folded normal form is denoted PrPC (for *Common or Cellular)*

It is encoded by the *PRNP gene on chromosome 20*

- Is water-soluble and proteinase K-sensitive
- Also expressed widely on the cells of immune system

Pathogenesis

- Hallmark of all prion diseases is that they result from aberrant metabolism and lead to accumulation of the prion protein
- PrPC → PrPSc (the abnormal disease-causing isoform,for *Scrapie, after one of the diseases first linked to prions and* neurodegeneration)
- Involves a conformation change
  - a helical content diminishes
  - amount of β sheet increases
- Protein X may facilitate β sheet formation

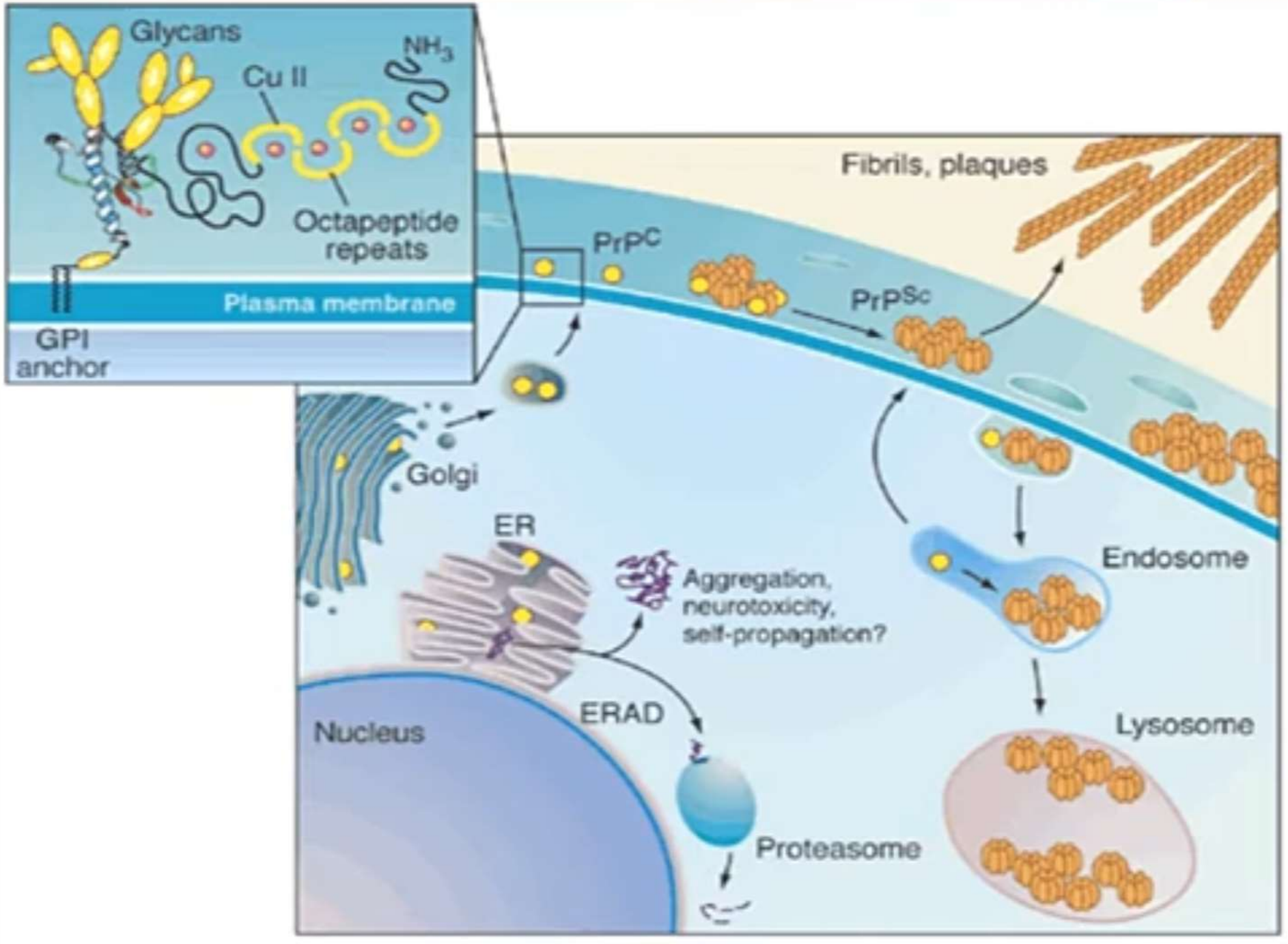


Source: *Prion Biology and Diseases*, Cold Spring Harbor Laboratory Press, New York, 1999

Fearure	PrPc	PrPsc
Full form	Prion protein cellular or common	Prion protein Scrapie
	Normal isoform of prion protein present in man / animals	Prion protein that causes disease in man / animal
Srructure	Elongated polypeptide, rich in – alpha helix and has little beta structure	- Globular polypeptide - less alpha helix but more beta structure
Location	Anchored to cell membrane	Cytoplasmic vesicles
Protease K	Sensitive	Resistant

Spongiform degeneration

- Initially, intracytoplasmic vacuoles (1-5µm) appear in neurons
- As the disease progresses, vacuolization becomes more pronounced
- The cortical neurophil develops a spongy appearance, hence the term **spongiform encephalopathy**
- Cerebral cortex, putamen, caudate nucleus, thalamus & molecular layer of cerebellum
- **Astrocytic gliosis**
- **Neuronal loss**


Glycans
Cu II
NH_3
Octapeptide repeats
Plasma membrane
GPI anchor
Fibrils, plaques
PrP^C
PrP^{Sc}
Golgi
ER
Aggregation, neurotoxicity, self-propagation?
Endosome
Nucleus
ERAD
Lysosome
Proteasome

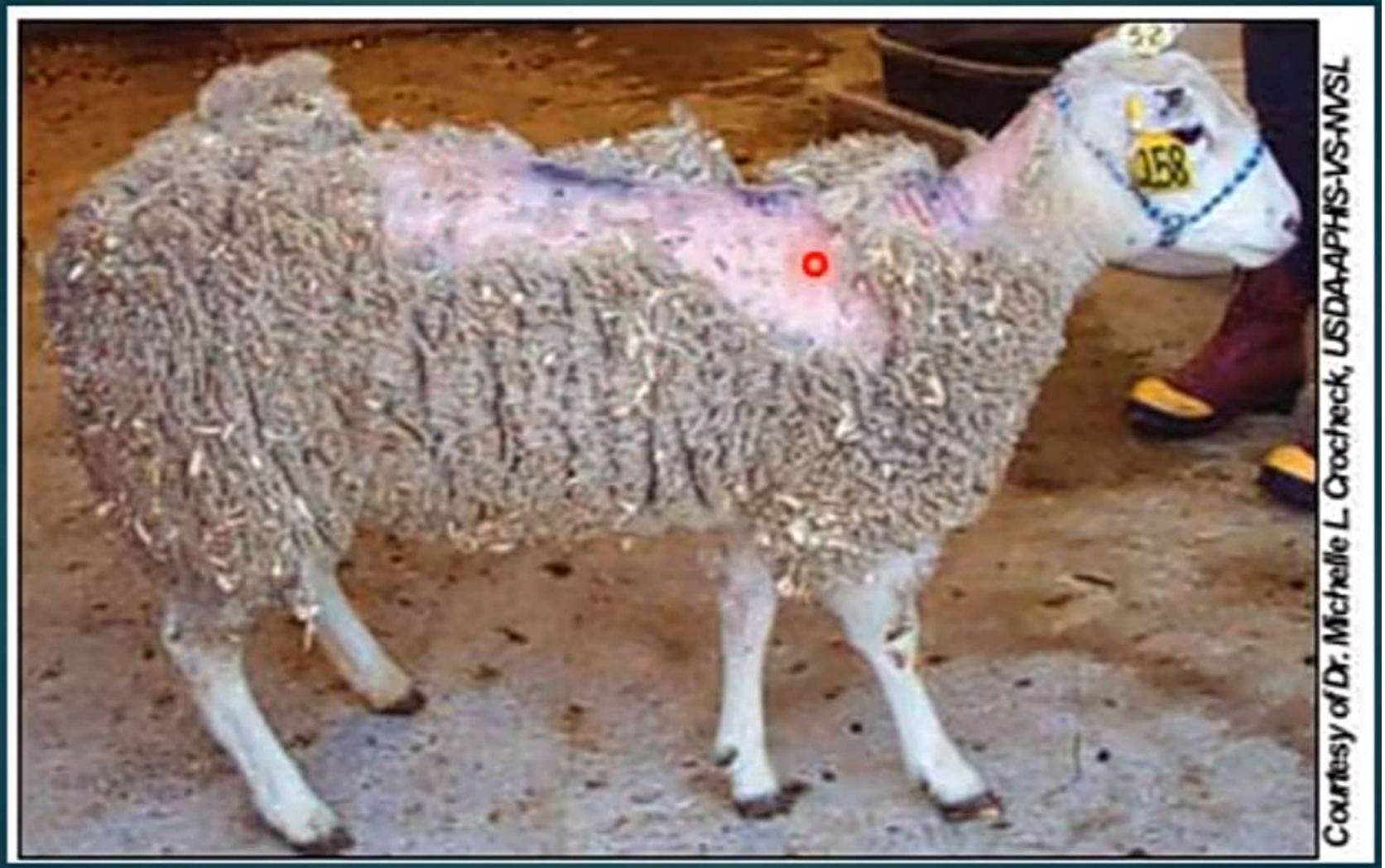
Prion diseases

- Prions cause diseases that we call **Transmissible spongiform encephalothies(TSE)** in both humans & animals
- **Animals**
  - Bovine spongiform encephalopathy (BSE)
  - Scrapie in sheep and goats
  - Transmissible mink encephalopathy
  - Chronic wasting disease of deer
- **Humans**
  - Creutzfeldt-Jacob disease (CJD)
  - Fatal familial insomnia (FFI)
  - Gerstmann-Straussler syndrome (GSS)
  - Kuru

Scrapie

- Fatal, degenerative disease that affects the nervous systems of sheep, goats.
- Identified in 18th century (1732)
- Does not appear to be transmissible to humans.
- It causes an itching sensation in the animals, so scraping themselves against trees and rocks ,hence the name Scrapie is given.
- Excessive lip-smacking, altered gaits, and convulsive collapse.

Scrapie



MAD COW DISEASE

Scientific Name: Bovine spongiform encephalopathy (BSE)

- It is found on any type of cloven hoofed animals such as: pigs, sheep, and cattle
- Spread to cattle: Sheep with Scrapie used in Meat and Bone Meal (MBM) – known as "Offal" MBM fed to cattle

Human infections

Kuru (tremor)

- Was identified in 1957 in Fore Tribe in New Guinea
- Characterised by
  - progressive cerebellar ataxia, tremers
  - Terminates fatally in 3-6 months
  - IP- 5-10 years
  - Infections believed to be due to cannibalism
  - A tribal custom of eating dead bodies of relatives after ritual non sterilising cooking
  - following the abolition of cannibalism , the disease has disappeared

Creutzfeldt-Jacob disease (CJD)

- CJD is the most common form of human prion diseases, is a subacute presenile encephalopathy, with progressive incoordination & dementia, ending fatally in a year
- Initially described by Jacob in 1921
- **Sporadic (sCJD) - most common (85-90%)**
- **Familial (fCJD) - 15%**
- **Iatrogenic (iCJd)- <1%**

Iatrogenic CJD

- **Sources :**
  - improperly sterilized depth electrodes
  - transplanted corneas
  - human growth hormone
  - dura mater grafts
  - gonadotropin derived from cadaveric pituitaries
  - surgical instruments

Gerstmann-Straussler-Scheinker disease

- Autosomal dominant (102 codon, proline to leucine)
- Occurs typically in 4th-5th decade
- Present with a slowly progressive limb and truncal ataxia, as well as dementia
- Prominent involvement of the brainstem degeneration
- Death occurs 3-8 years following presentation
- The neuropathology of GSS is remarkable that extensive and invariable amyloid deposition occurs
- Along with the typical spongiform change,

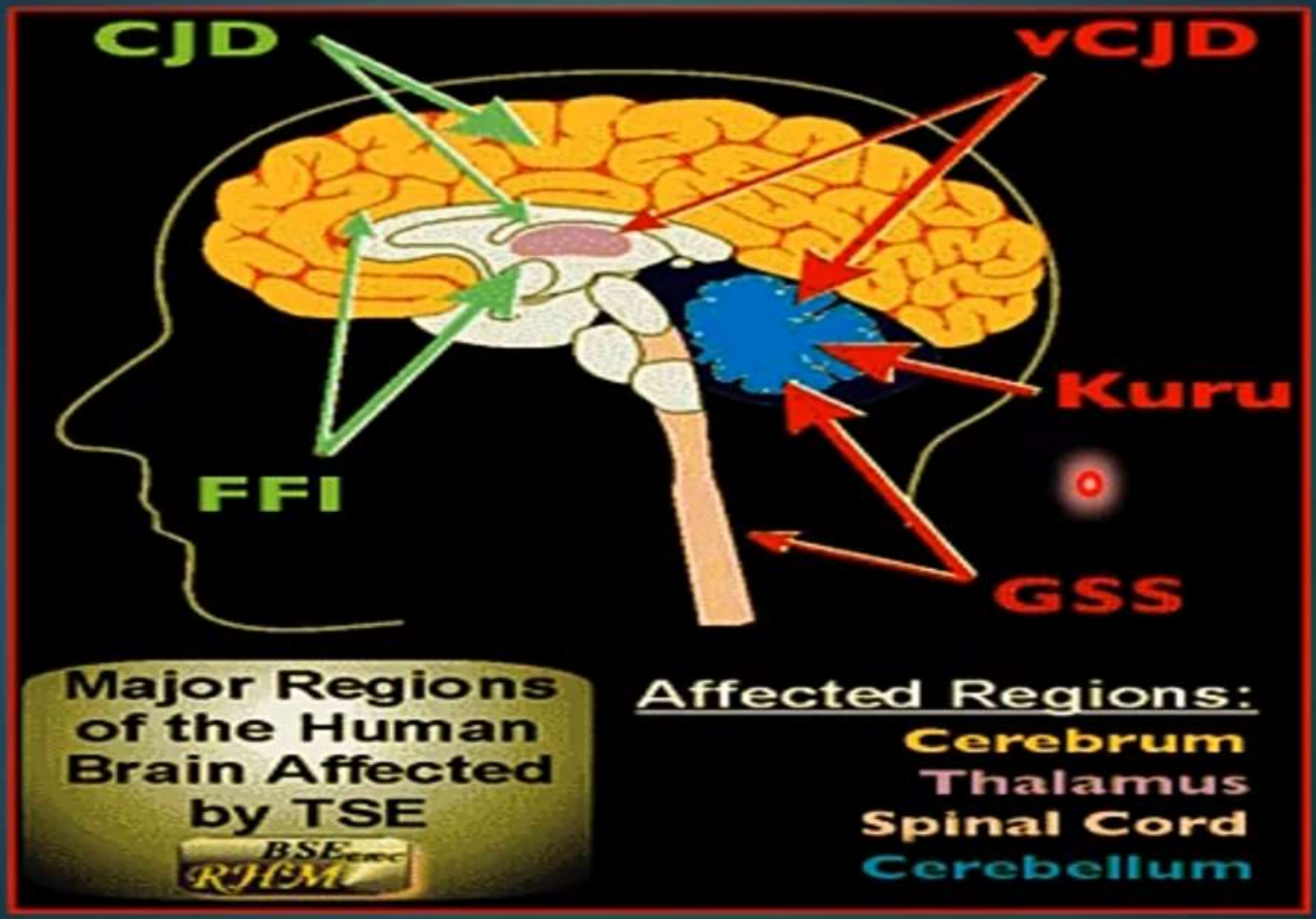
Fatal familial insomnia

- Age of onset is variable, ranging from 18-60 years
- Presentation:
  - intractable insomnia
  - dementia
  - motor paralysis
  - dysautonomia (ie, hyperthermia, hypertension,
  - tachypnea, hyperhydrosis)

Progressive multifocal Leucoencephalopathy

- is a rare demyelinating disease in elderly persons
- Whose immune response is impaired by immuno suppression & malignancy
- JC virus (member of Polyomaviridae)
- About 5% of patients with AIDS
- Demyelination of CNS occurs due to oligodendrocyte infection by polyomavirus.
- Progressive deterioration of motor function, vision and speech.
- Death occurs in 3-4 months
- Polyoma virus has been demonstrated by electron microscopy in brain biopsy of

Different prions affect different parts of brain



Laboratory Diagnosis

- Clinical and investigative features, which are included in the diagnosis criteria, may be indicative of the diagnosis of prion diseases but are never definitive
- **Clinical: features depends on the sites of CNS involved**
- **Investigations:**
  - **Conformation dependent immunoassay (CDI)**
  - Extremely sensitive & quantitative
  - Useful for both ante & post mortem detection of prions (PrPsc)

- **MRI brain scan**
  - Shows high signal intensity in
    - basal ganglia 70% of cases of sporadic CJD
    - posterior thalamus in 90% of cases of vCJD
- **DNA sequencing**
  - Can be done on extracts from blood, brain, and other tissues
  - Detects :

    Mutations of the *PRNP gene*

Brain biopsy

A definitive diagnosis is made by microscopic examination of brain tissue

	sCJD	vCJD	FFI	GSS
Spongiform degeneration	++	++	+/-	++
Gliosis	+	+	+	+
Amyloid plaques	+	++	+	+++

CSF

- Protein and glucose concentration is normal
- No pleocytosis
- Elevation of stress **protein 14-3-3: most useful CSF marker of CJD**
- **Protein 14-3-3 is elevated in**

- 90% of cases of sporadic CJD
- 50% of vCJD cases

- This protein is also elevated in patients with encephalitis, cerebral infarction, and other conditions

- **EEG (electroencephalogram)**
  - Shows a typical periodic pattern
  - In late stage, high voltage triphasic sharp discharges seen
- **CT scan**
  - Normal/cortical atrophy
- **Western blotting of PrPsc**
  - Following proteinase K digestion reveals electrophoretic patterns that identify different prion strains
  - PrP 27-30 is protease resistant core of PrPsc

- PrPsc can be detected in
  - -brain tissue extracts by **ELISA**
  - -tissue sections by **immunohistochemistry**
- As PrPsc not uniformly distributed throughout the CNS, absence of PrPsc in limited sample like brain biopsy does not rule out prion disease

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Slow virus by Science Direct

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https://link.springer.com

Unit III

TRANSPLANTATION IMMUNOLOGY

Presented by

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Immunologic basis of Graft rejection:

Graft rejection is an immunogenic response displaying the attribute of specificity,memory and non-self recognition.

There are three major types of rejection reaction -

1)Hypercute rejection -mediated by preexisting host antibodies to graft antigens

2)acute graft rejection -TH cell and CTLs mediate tissue damage

3)Chronic rejection -which involves both cellular and humoral immune components

A) Autograft- Is self transferd from one body site to another in the same individual.
Ex. Transferring healthy skin to burned area in burn patients and use of Heathy blood vessels to replace blockd coronary artery
B)Isograft -Is tissue transferd between genetically identical individuals.
Ex. In inbred strains of mice, an isograft can be performed from One mouse to another syngeneic mouse.
In humans, an isograft can be performed between genetically identical twins

C) Allograft - Is tissue transfered between genetically different members of the same species.

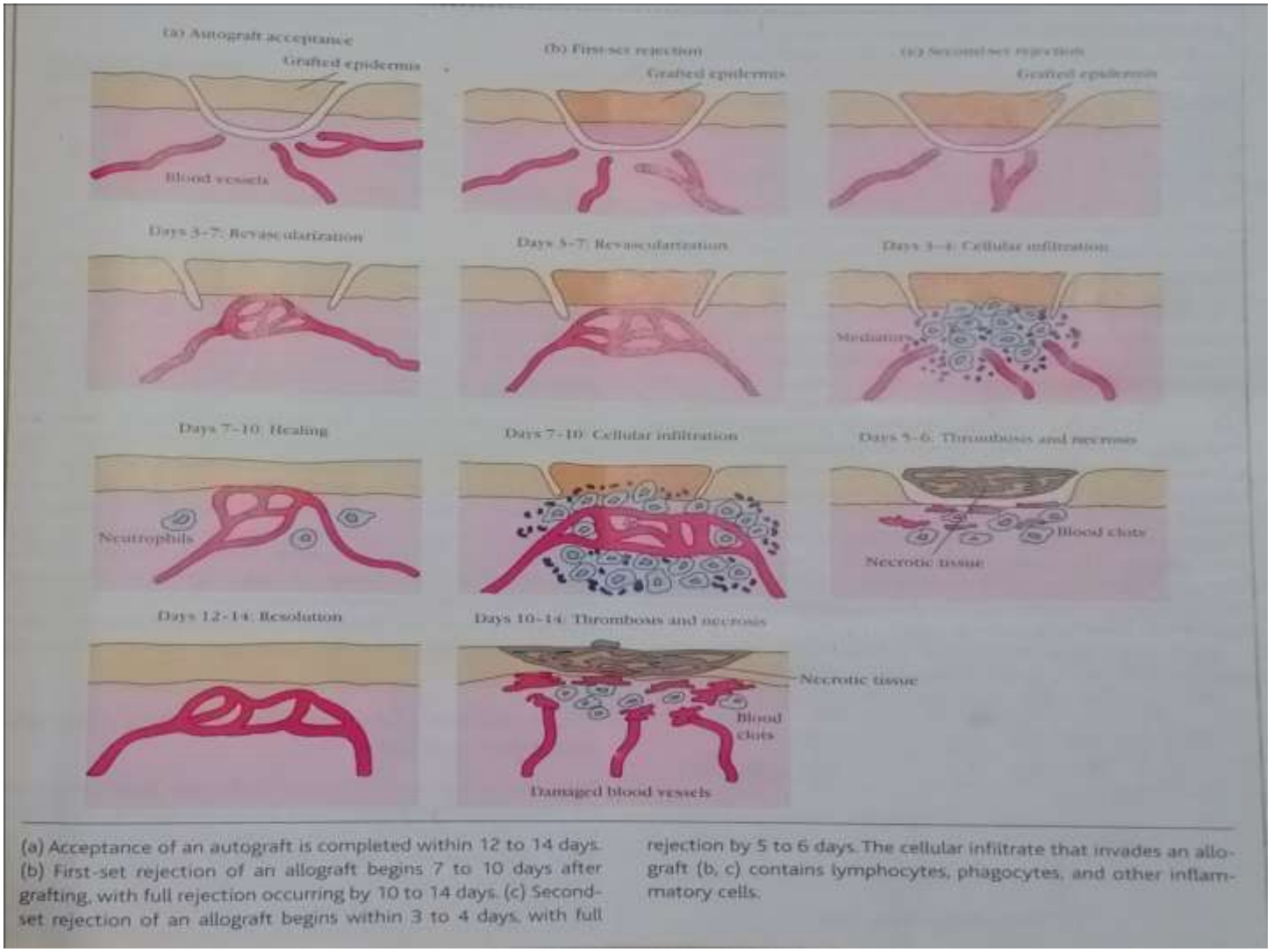
Ex-In mice, an allograft is performed by transferring tissue or an organ from one strain to another.

In humans, organ graft from one individual to another are allograft Unless the donor and recipient are identical twins.

D) Xenograft - Is tissue transferred between different species.

Ex. The graft of a baboon heart into a human.

Allograft rejection displays specificity and memory:



(a) Acceptance of an autograft is completed within 12 to 14 days. (b) First-set rejection of an allograft begins 7 to 10 days after grafting, with full rejection occurring by 10 to 14 days. (c) Second-set rejection of an allograft begins within 3 to 4 days, with full rejection by 5 to 6 days. The cellular infiltrate that invades an allograft (b, c) contains lymphocytes, phagocytes, and other inflammatory cells.

T-cell play a key role in allograft rejection:

In the early 1950s, Avrion Mitchison showed in adoptive transfer experiment (in which host lymphocytes are killed with X-ray and donor immune cell are introduced) that lymphocytes, but not serum antibody, could transfer allograft immunity.

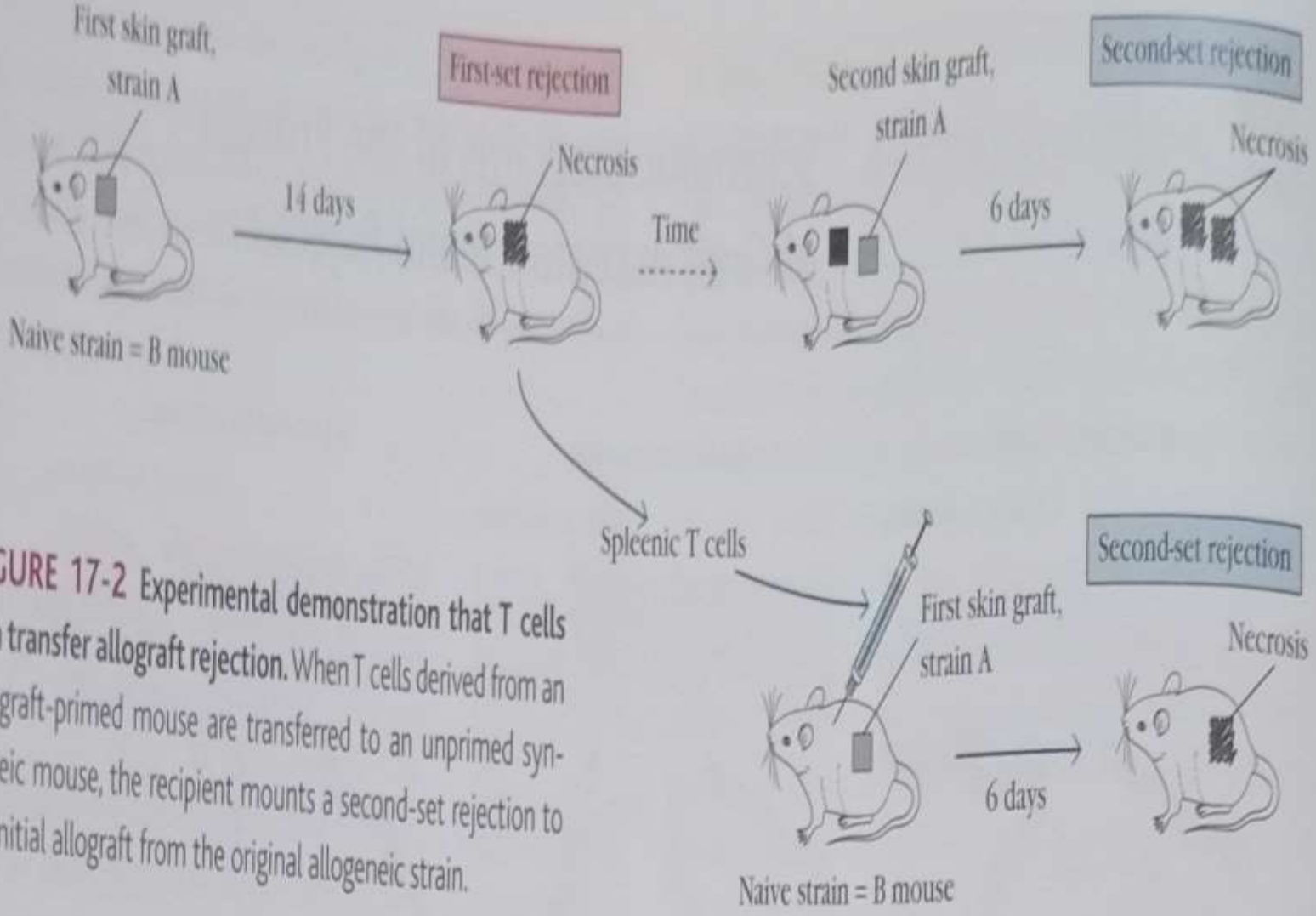


FIGURE 17-2 Experimental demonstration that T cells can transfer allograft rejection. When T cells derived from an allograft-primed mouse are transferred to an unprimed syngeneic mouse, the recipient mounts a second-set rejection to an initial allograft from the original allogeneic strain.

Analysis of the T cells subpopulation involved in allograft rejection has implicated both CD4+ And CD8+

In one study, mice were injected with monoclonal antibodies to deplete one or both types of T cells and then the rate of graft rejection was measured.

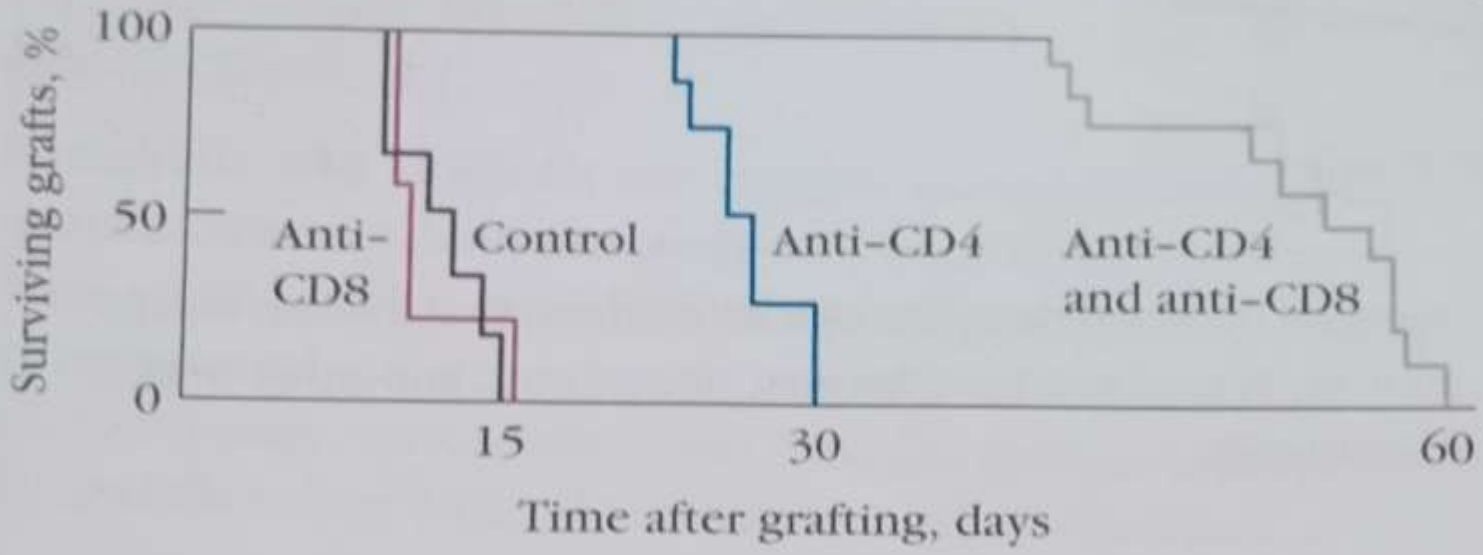


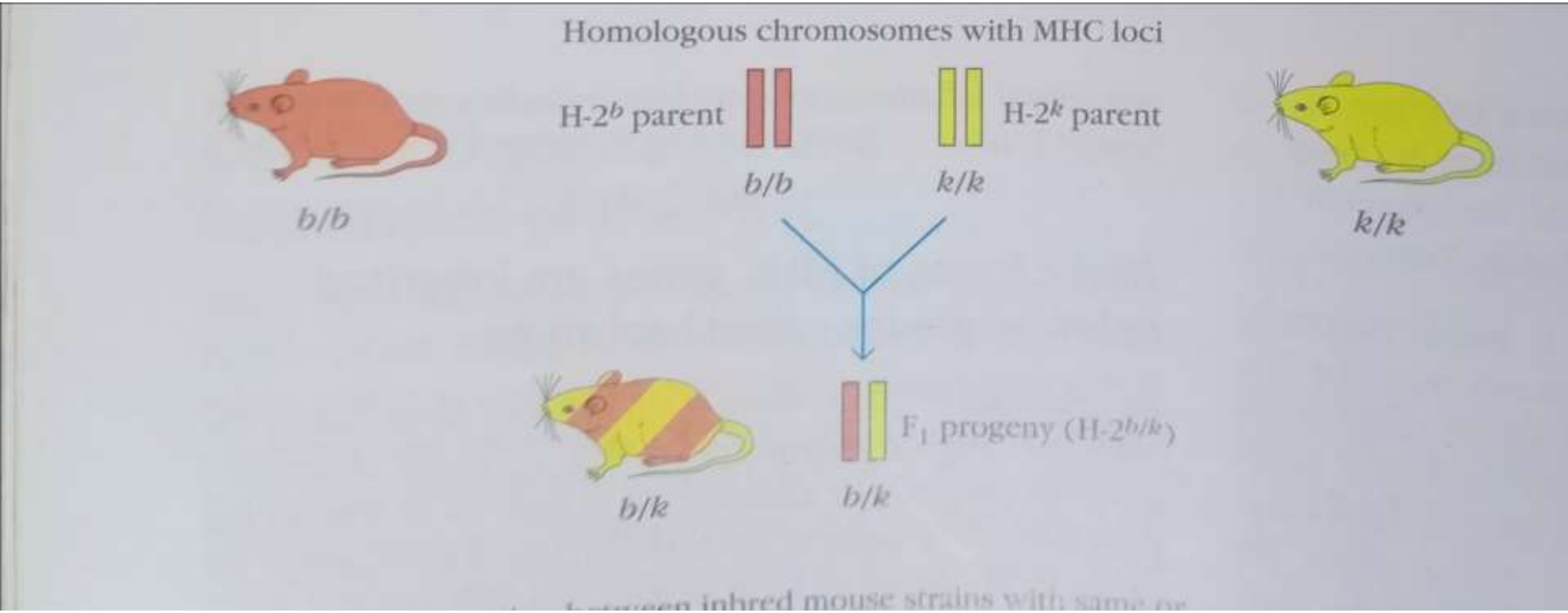
FIGURE 17-3 The role of CD4$^+$ and CD8$^+$ T cells in allograft rejection is demonstrated by the curves showing survival times of skin grafts between mice mismatched at the MHC. Animals in which the CD8$^+$ T cells were removed by treatment with an anti-CD8 monoclonal antibody (red) showed little difference from untreated control mice (black). Treatment with monoclonal anti-CD4 (blue) improved graft survival significantly, and treatment with both anti-CD4 and anti-CD8 antibody prolonged graft survival most dramatically (green). *[Adapted from S. P. Cobbold et al., 1986,* Nature **323**:*165.]*

- **Similar antigenic profile foster allograft acceptance**
  Tissue that are antigenically similar are said to be histocompatible, such tissue do not induce an immunological response that lead to tissue rejection. Tissue that display significant antigenic differences are histoincomatible and induce an Immune response that lead to tissue rejection.

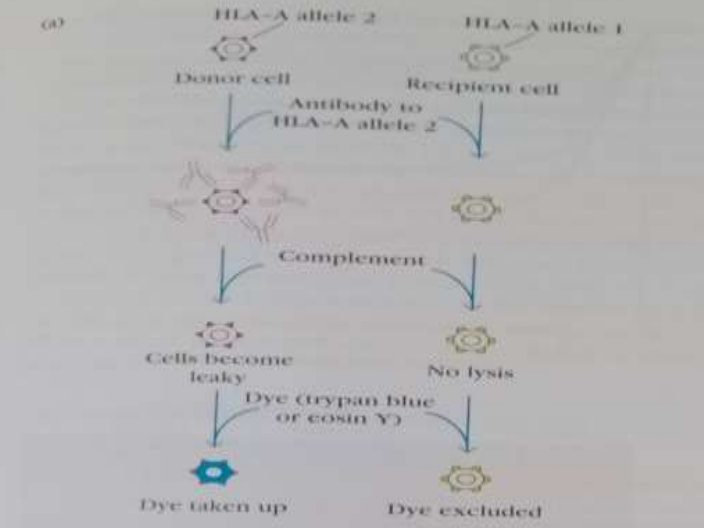
  The various **antigens** that determine histocompatibility are encode by more than 40 different loci, but the loci responsible for the most vigorous allograft rejection reaction are located within the major histocompatibility complex

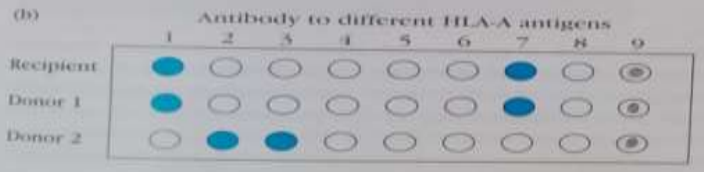
The organization of the MHC called the H -5 complex in mice and HLA complex in humans.

Because the MHC loci are closely linked, they are usually inherited as a complete set, called a haplotype, from each parent.



Graft donor and recipient are typed for RBC and MHC antigens





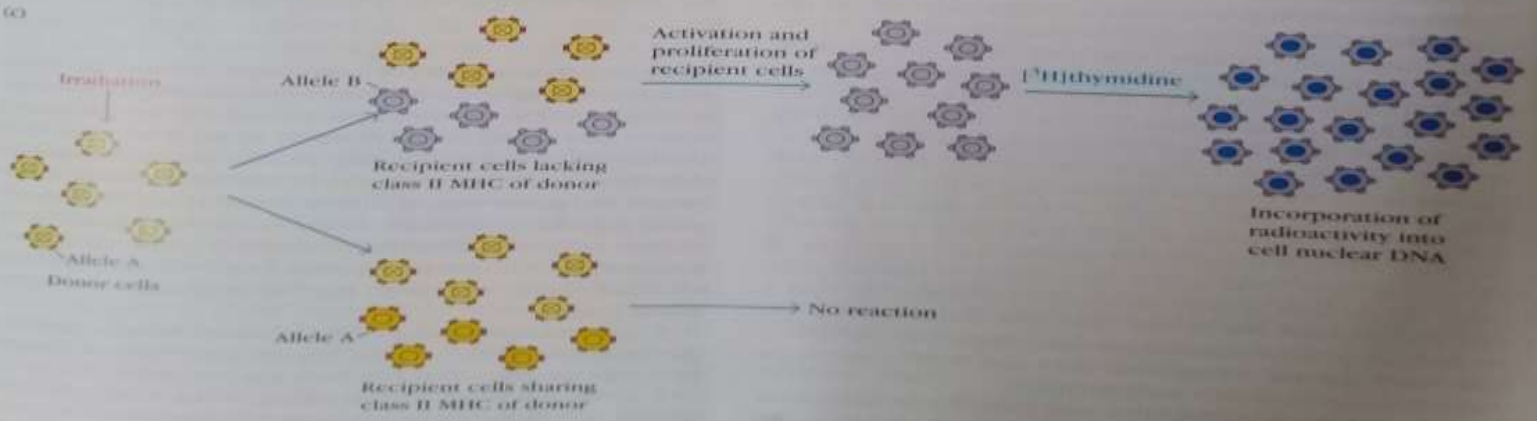


FIGURE 17-4 Typing procedures for HLA antigens. (a, b) HLA typing by microcytotoxicity. (a) White blood cells from potential donors and the recipient are added to separate wells of a microtiter plate. The example depicts the reaction of donor and recipient cells with a single antibody directed against an HLA-A antigen. The reaction sequence shows that if the antigen is present on the lymphocytes, addition of complement will cause them to become porous and unable to exclude the added dye. (b) Because cells express numerous HLA antigens, they are tested separately with a battery of antibodies specific for various HLA-A antigens. Here donor 1 shares HLA-A antigens recognized by antisera in wells 1 and 7 with the recipient, whereas donor 2 has none of the HLA-A antigens in common with the recipient. (c) Mixed lymphocyte reaction to determine identity of class II HLA antigens between a potential donor and recipient. Lymphocytes from the donor are irradiated or treated with mitomycin C to prevent cell division and then added to cells from the recipient. If the class II antigens on the two cell populations are different, the recipient cells will divide rapidly and take up large quantities of radioactive nucleotides into the newly synthesized nuclear DNA. The amount of radioactive nucleotide uptake is roughly proportional to the MHC class II differences between the donor and recipient lymphocytes.

HLA TISSUE TYPING

- The blood group Antigen and Major Histocompatibility Antigen are responsible for graft acceptance ,rejection process in Transplantation
- Initially ,donor and recipient are screened for ABO blood group compatibility.
- The blood group Antigen are expressed on RBCs, Epithelial cells and Endothelial Cell
- Various Tissue Typing procedure are developed to identify the antigen and to screen the potential donor and the recipient cells
- They are

1.MICROCYTOTOXICITY TEST

2.MIXED LYMPHOCYTE REACTION (MLR)

1.MICROCYTOTOXICITY TEST

White blood cells from potential Donor and Recipient, are distributed into a series of wells on a Microtiter plates.

Antibodies specific for Various class I & II MHC allele are added to different wells

After incubation Complement is added to the wells

Cytotoxicity is assesed by the uptake or exclusion of various dye (Trypan blue or Eosin Y)by the cells.

INFERENCE

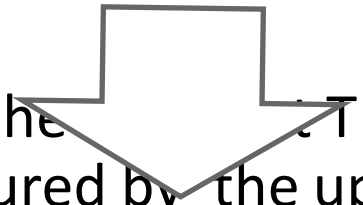
- If The WBC express the MHC allele, For which a particular MCA is specific then the cell will be lysed upon addition of Complement and these dead cells will be taken up the dye such as Trypan blue or Eosin Y

- The HLA Tissue typing based on Antibody mediated cytotoxicity, thus indicate the presence or absence of various MHC alleles.

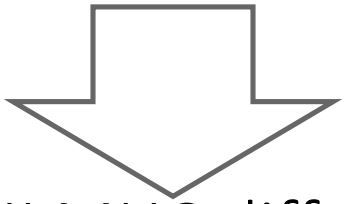
MIXED LYMPHOCYTE REACTION

- Even when a fully HLA-compatible donor is not available, transplantation may be successful.
- In this situation, a one way mixed -lymphocytes reaction (MLR) can be used to qualify the degree of class II MHC compatibility between potential donor and recipient.

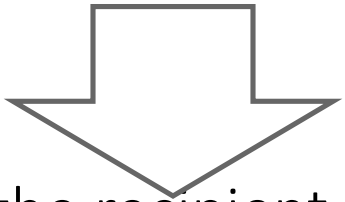
Lymphocytes from a potential donor that have been X -irradiated or treated with mitomycin C serve as the stimulator cell, & lymphocytes from the recipient serve as responder cell.



Proliferation of the [illegible] T cells, which indicates T-cell activation, is measured by the uptake of (3H) thymidine into cellular DNA.



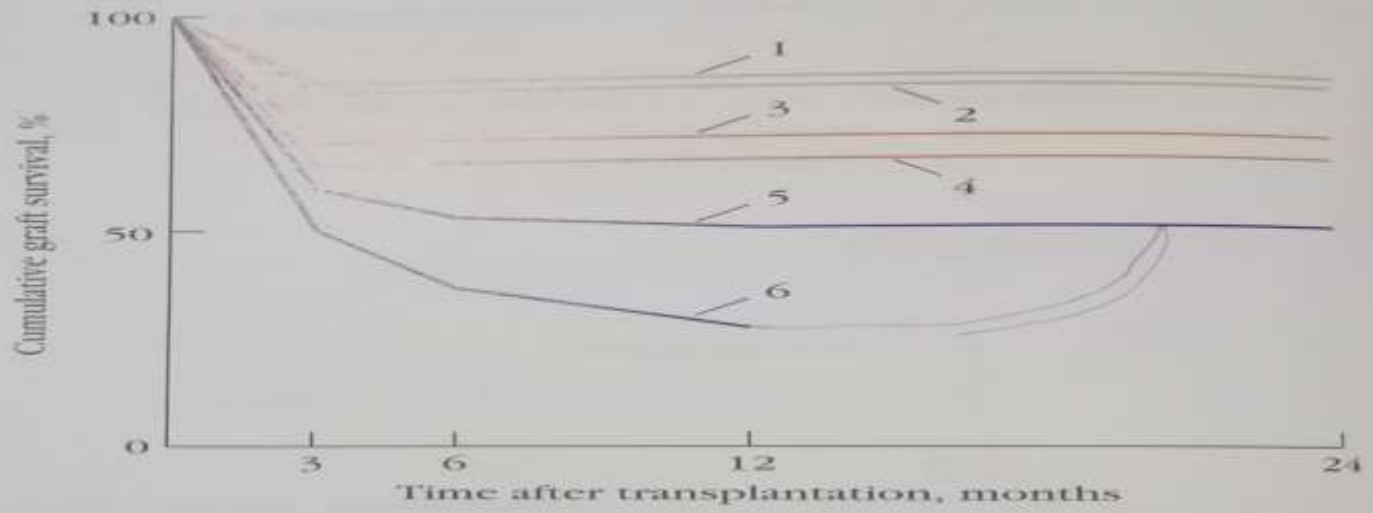
The greater the class II MHC difference between the donor and recipient cell, the more [3H]thymidine uptake will be observed in an MLR assay.



Intense proliferation of the recipient Lymphocytes indicates a poor progenesis for graft survival.

- The **Advantage** of the MLR Over microcytotoxicity typing is that it gives a better indication of the degree of TH cell activation generated in response to the class II MHC antigens of the potential graft.

Disadvantages -take several days to run the assay.



Curve no.	HLA mismatches (no.) Class I	Class II
1	0	0
2	1 or 2	0
3	3 or 4	0
4	0	1 or 2
5	1 or 2	1 or 2
6	3 or 4	1 or 2

FIGURE 17-5 The effect of HLA class I and class II antigen matching on survival of kidney grafts. Mismatching of one or two class I (HLA-A or HLA-B) antigens has little effect on graft survival. A single class II difference (line 4) has the same effect as three or four differences in class I antigens (line 3). When both class I and class II antigens are mismatched, rejection is accelerated. *[Adapted from T. Moen et al., 1980,* New England Journal of Medicine **303**:850.*]*

- Cell membrane graft rejection occurs in two stages
  Graft rejection is caused Principally by a cell mediated immune response to alloantigens expressed on cell of the graft. Both delayed type hypersensitive and cell mediated cytotoxycity reaction can have been implemented.
  The process of graft rejection can be divided into two stages
  :1)**sensitization phase,** in which antigen reactive lymphocytes of the recipient proliferate in response to alloantIgen On the graft and
  2)an **effector stage**, in which immune destruction of the graft take place

Sensitization stage:

- During the sensitization phase, $CD4^+$ and $CD8^+$ T cells recognize alloantigens express on the cell of the foreign graft and proliferate in response.
- Both major and minor histocompatibility alloantigens can be recognized. The response to major histocompatibility antigens involves recognition of both the donor MHC molecule and an associated peptide ligand in the cleft of the MHC colecule.
- A host T_H cell becomes activated when it interacts with an antigen-presenting cell that both expresses an appropiate antigenic ligand-MHC molecule complex and provides the requisite co-stimulatory signals.

- Depending on the tissue, different populations of cells within a graft may function as APCs. Because dendritic cells are found in most tissues and because they constitutively express high level of class II MHC molecules, dendritic cells generally serve as the major APC in grafts.
- APCs of host origin can also migrate into a graft and endocytose the foreign alloantigens and present them as processed peptide together with self MHC molecules to T_H cells.
- In some organ and tissue grafts a population of donor APCs called passenger leukocytes has been shown to migrate from graft to the regional lymph nodes.

- These passenger leukocytes are dendritic cells, which express high levels of class II MHC molecules. But passenger leukocytes are not only one involved in immune stimulation.
- Recognition of the alloantigens expressed on the cells of a graft induces vigorous T-cell proliferation in the host.
- Both dendritic cells and vascular endothelial cells from an allogeneic graft induce host T-cell proliferation.
- The major proliferating cell is $CD4^+$ T cell. This amplified population of activated T_H cell is thought to play the central role in inducing the various effector mechanism of allograft rejection.

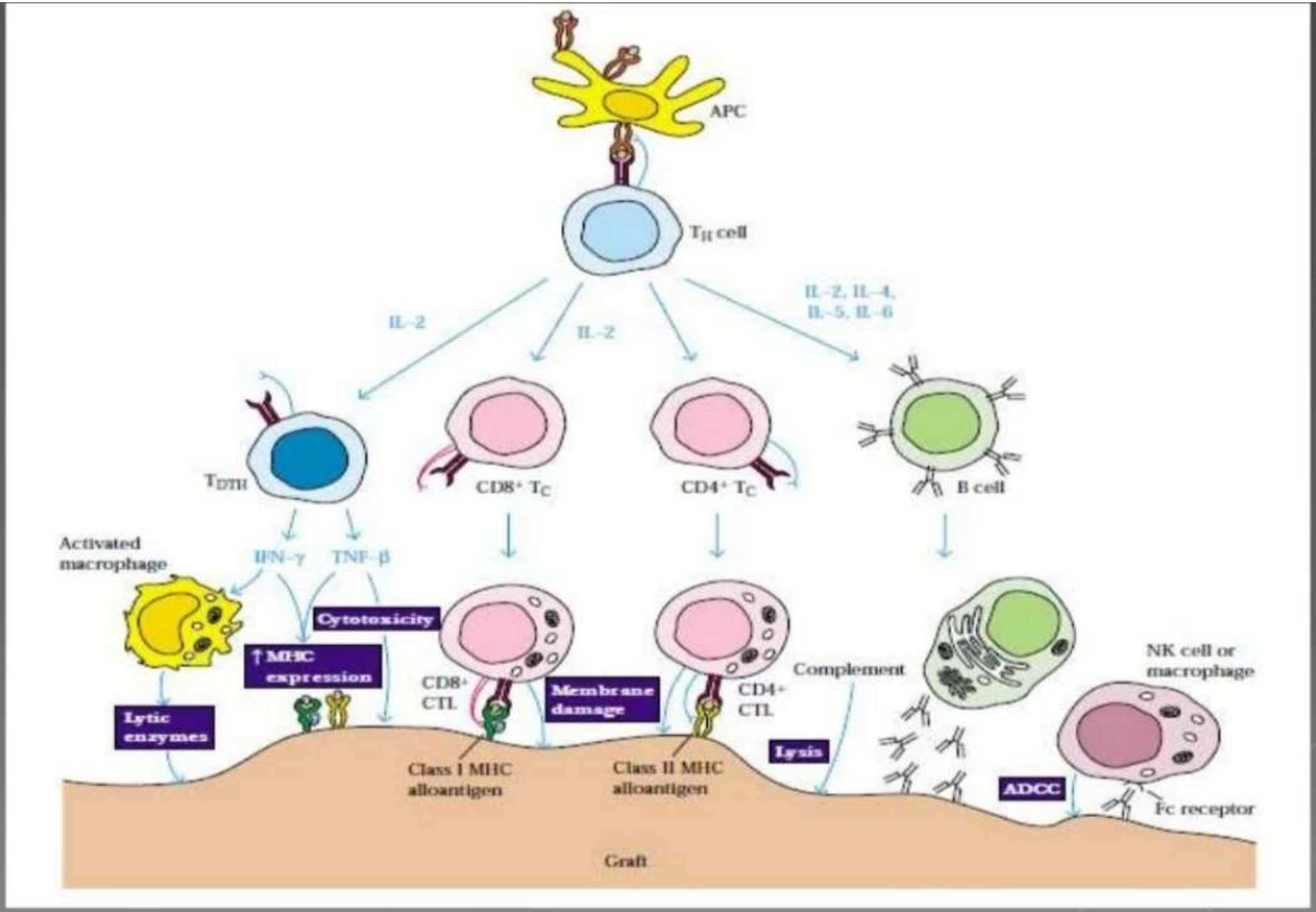
Effector stage:

- A variety of effector mechanisms participate in allograft rejection. The most common are cell mediated reactions involving delayed type hypersensitivity and CTL(cytotoxic T lymphocyte) mediated cytotoxicity ; less common mechanisms are antibody-plus-compliment lysis and destruction by antibody dependent cell mediated cytotoxicity (ADCC).
- The hallmark of graft rejection involving cell mediated reactions is an influx of T-cell and macrophages into the graft.
- Infiltration can happen by delayed type hypersensitivity response also in which cytokines

produced by T_{DTH} cells promote macrophage infiltration.

- Recognition of foreign class I alloantigens on the graft by host $CD8^+$ cells can lead to CTL mediated killing. In some cases $CD4^+$ T cells that function as class II MHC restricted cytotoxic cells mediated graft rejection.
- In each of these mechanisms cytokines secreted by T_H cells play a central role. For example IL-2, IFN-γ, and TNF-β are important mediator for graft rejection.
- IL-2 promotes T-cell proliferation and necessary for generation of effector CTLs.
- IFN-γ is central to the development of a DTH response, promoting the influx of macrophages into the graft.

- TNF-β has been shown to have a direct cytotoxic effect on the cells of a graft.



Effector Mechanism

Clinical manifestation of graft rejection

- **Graft rejection reaction have various time courses depending on the type of tissue or organ grafted and the immune response involved.**
- hyperacute rejection reactions occur within the first 24 hours after transplantation .
- Acute rejection reactions usually begin in the first few weeks after transplantation .
- Chronic rejection reactions can occur from months to years after transplantation .

Pre existing recipient antibodies mediate hyperacute rejection:

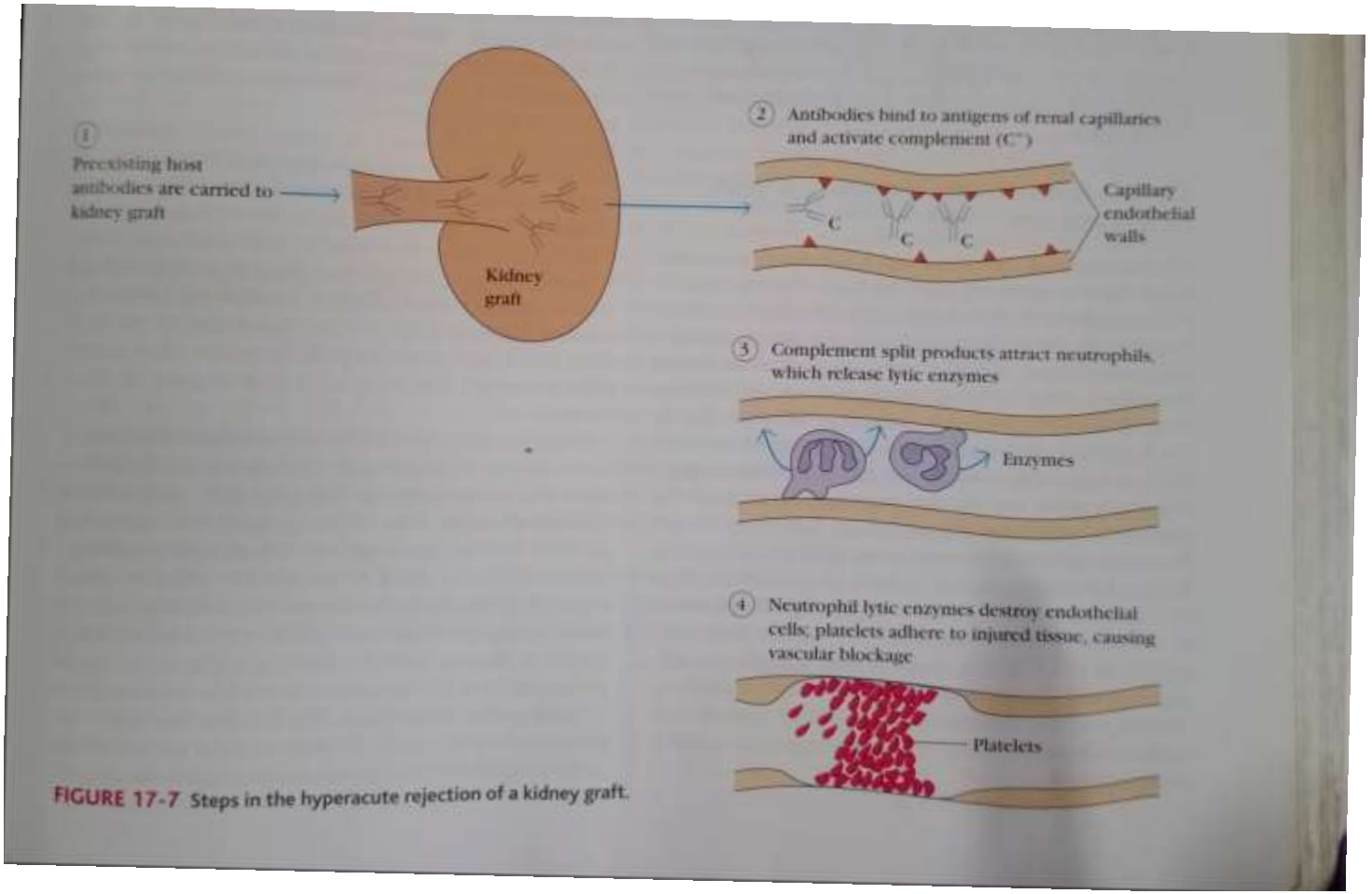


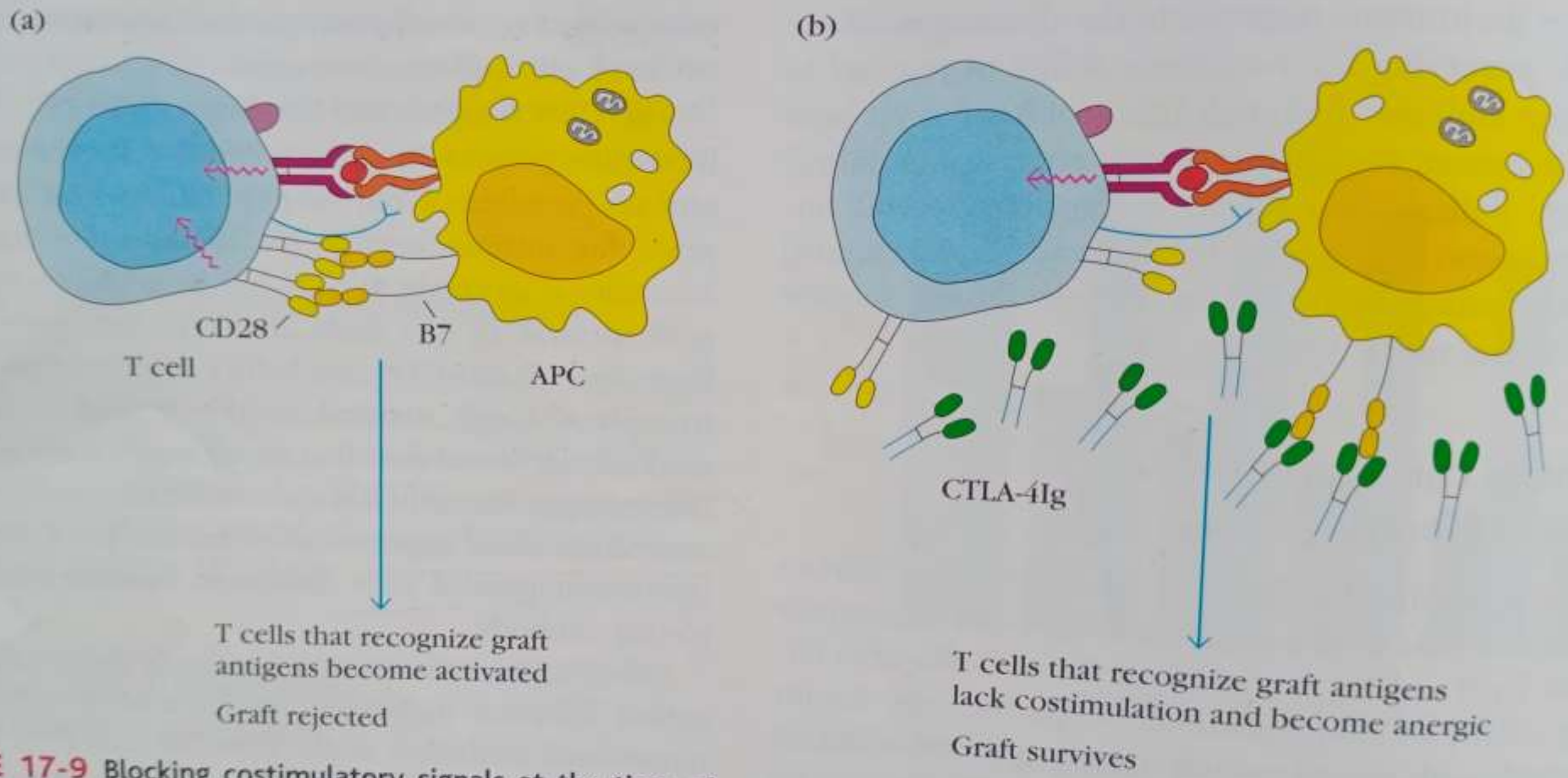
FIGURE 17-7 Steps in the hyperacute rejection of a kidney graft.

- In some cases the pre existing antibodies participating in hyperacute graft rejection maybe specific for blood group antigen in the grapft.
  If tissue typing and a b o blood group typing are performed prior to transplantation this pre existing antibodies can be detected and graft that would result in hyperacute rejection can be avoided .
  In addition to hyperactive rejection mediated by pre existing antibodies, there is less frequent form of rejection term **accelerated rejection** caused by antibodies that are produced immediately after transplantation .

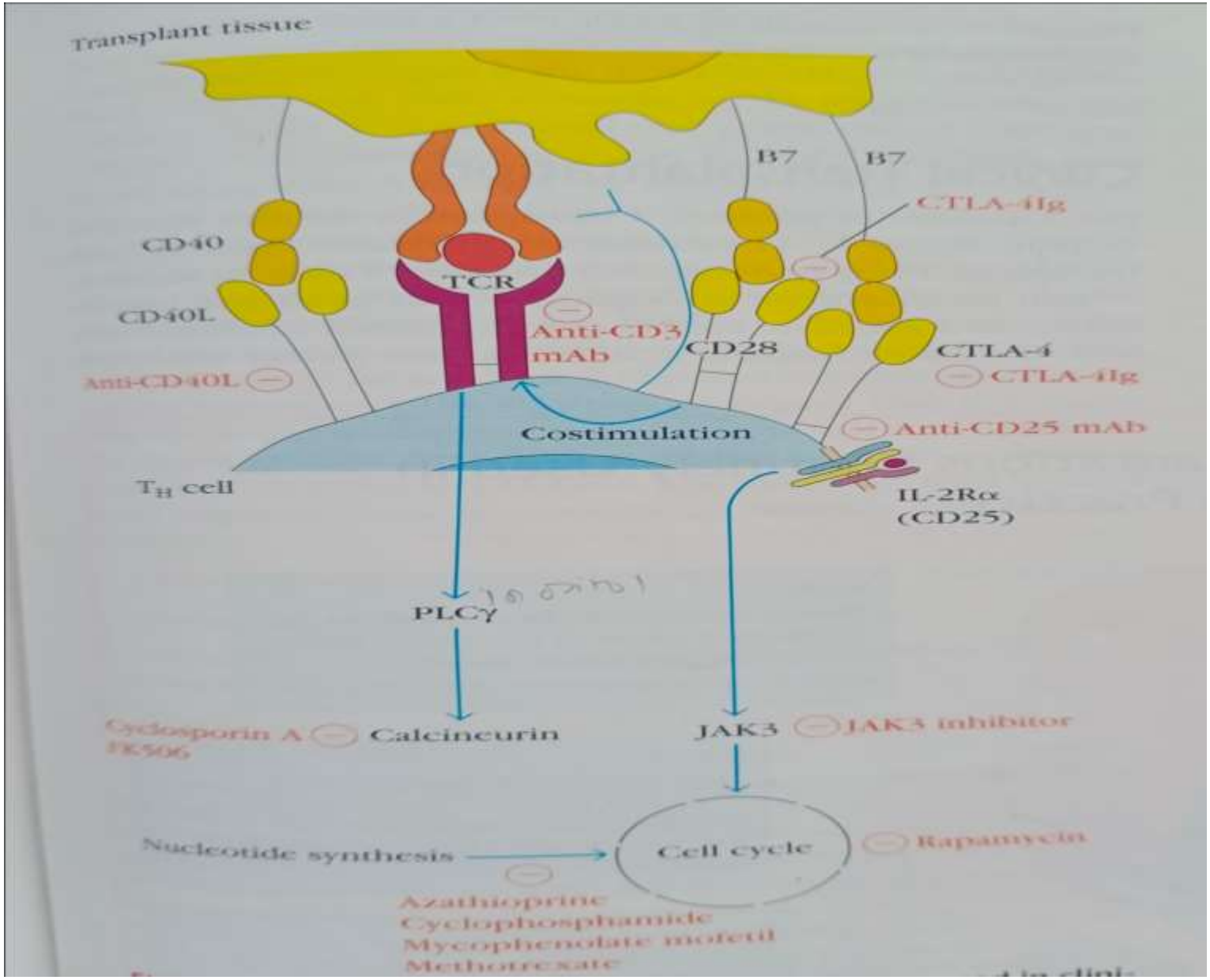
Acute rejection is mediated by T cell responses
Cell mediated allograft rejection manifest as an acute rejection of the graft beginning about 10 days after transplantation histopathologicaI examination reveals A massive in filtration Of macrophages and lymphocytes at the siTe of tissue destruction Suggestive of Th cell activation And proliferation

- Chronic rejection occurs month or years post transplant
  The mechanism of chronic rejection include both Humeral and cell mediated responses by the recipient.
  Although the use of immunosuppressive drugs and the application of tissue typing methods to obtained optimum match of Donor and recipient and have dramatically increase the first years after engraftment, little progress has been made in long term survival.
  The use of immunosuppressive drug greatly increases the short term survival of the transplant but chronic reaction on is not prevented in most cases
  only about 50% of transplanted Kidneys are still functioning at 10 years after transplant.
  Chronic rejection reaction a difficult to manage with immunosuppressive drugs and may necessitate another transplantation

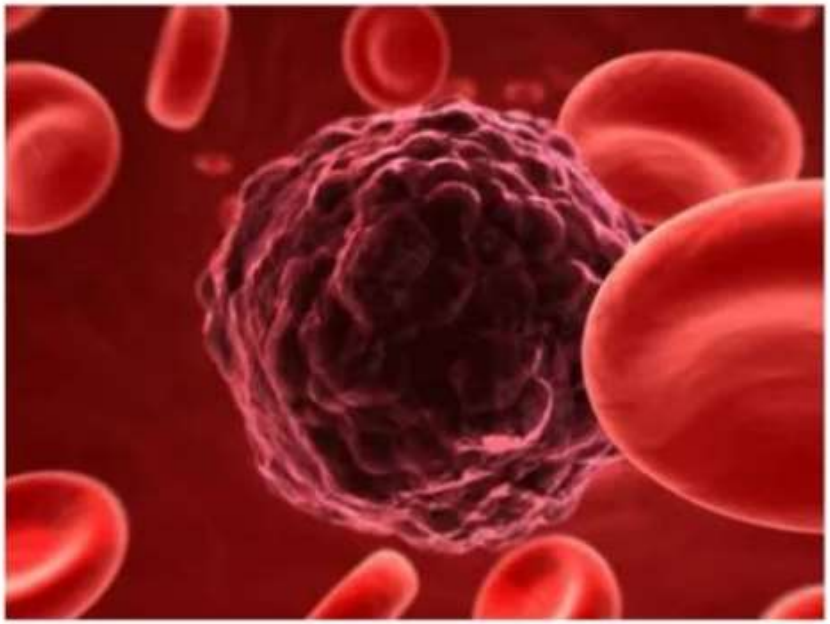
- **Specific immune supreesive therapy**
  Antibodies can suppress graft rejection response
  Blocking costimulatory signals can reduce anergy



RE 17-9 Blocking costimulatory signals at the time of plantation can cause anergy instead of activation of the T reactive against the graft. T-cell activation requires both the action of the TCR with its ligand and the reaction of costimula- receptors with their ligands (a). In (b), contact between one of the costimulatory receptors, CD28 on the T cell, and its ligand, B7 on APC, is blocked by reaction of B7 with the soluble ligand CTLA- The CTLA-4 is coupled to an Ig H chain, which slows its clearance f the circulation. This process specifically suppresses graft reject without inhibiting the immune response to other antigens.


Transplant tissue
B7
B7
CTLA-4Ig
CD40
TCR
CD40L
Anti-CD3 mAb
CD28
CTLA-4
Anti-CD40L
CTLA-4Ig
Costimulation
Anti-CD25 mAb
T_H cell
IL-2Rα (CD25)
PLCγ
Cyclosporin A FK506
Calcineurin
JAK3
JAK3 inhibitor
Nucleotide synthesis
Cell cycle
Rapamycin
Azathioprine
Cyclophosphamide
Mycophenolate mofetil
Methotrexate

TUMOR ANTIGENS



INTRODUCTION:

- Tumor antigen is an antigenic substance produced in tumor cells i.e., it triggers an immune reponse in host cells
- These are not membrane proteins.
- The sub discipline of tumour immunology involves the study of antigens on tumour cells and the immune response to these antigens. Two types of tumour antigens have been identified on tumour cells:

- TSTAs Tumour specific transplantation antigens
- TATAs Tumour associated transplantation antigens

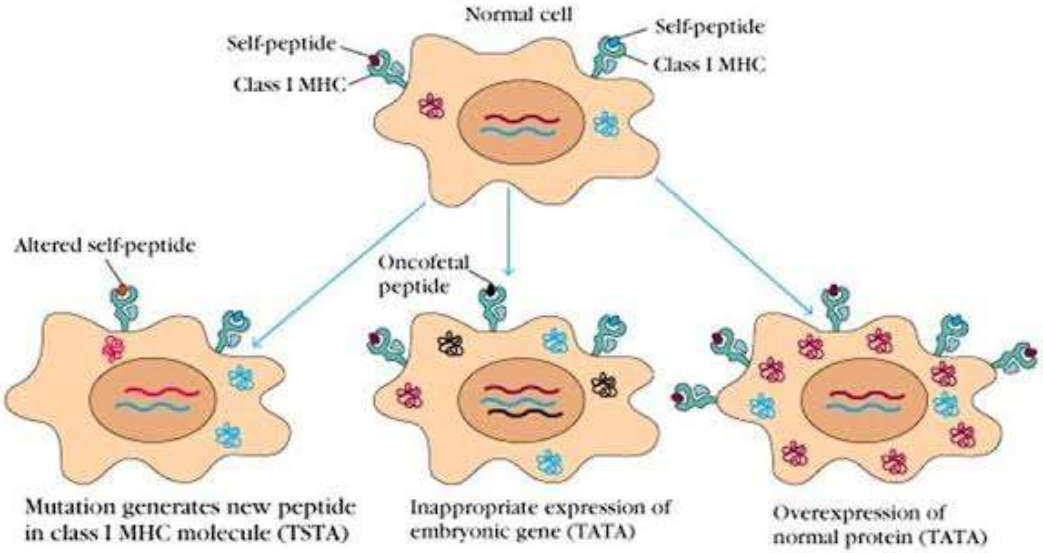
TSTAs

- Unique to tumour cells; that do not occur on normal cells in the body. They may result from **mutations** in tumour cells that generate altered cellular proteins, cytosolic processing of these proteins would **give rise to novel peptides** that are presented with Class 1 MHC molecules, including a cell mediated response by tumour specific CTLs
- Produced due to physical, chemical or viral mutagens.

TATAs

- Which are not unique to tumour cells, may be protein that are expressed on normal cells during foetal development when the immune system is immature and unable to respond but that normally are not expressed in the adult. **Reactivation of embryonic genes** that encode these proteins in tumour cells. Result in their expression on the fully differentiated tumour cells.

Tumor antigens



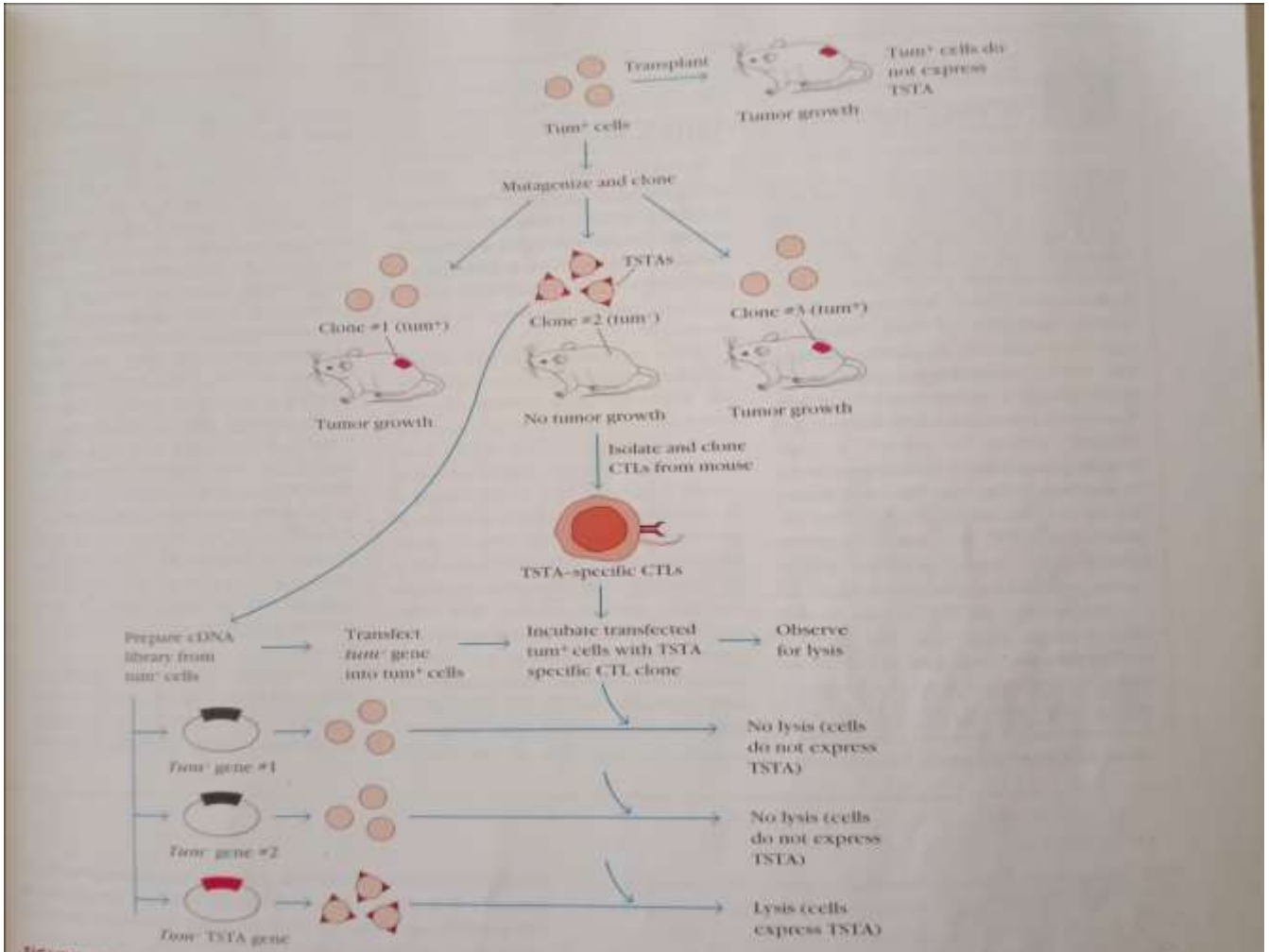


FIGURE 21-7 One procedure for identifying genes encoding tumor-specific transplantation antigens (TSTAs). Most TSTAs can be detected only by the cell-mediated rejection they elicit. In the first part of this procedure, a nontumorigenic (tum⁻) cell line is generated; this cell line expresses a TSTA that is recognized by syngeneic mice, which mount a cell-mediated response against it. To isolate the gene encoding the TSTA, a cosmid gene library is prepared from the tum⁻ cell line, the genes are transfected into tumorigenic tum⁻ cells, and the transfected cells are incubated with TSTA-specific CTLs.

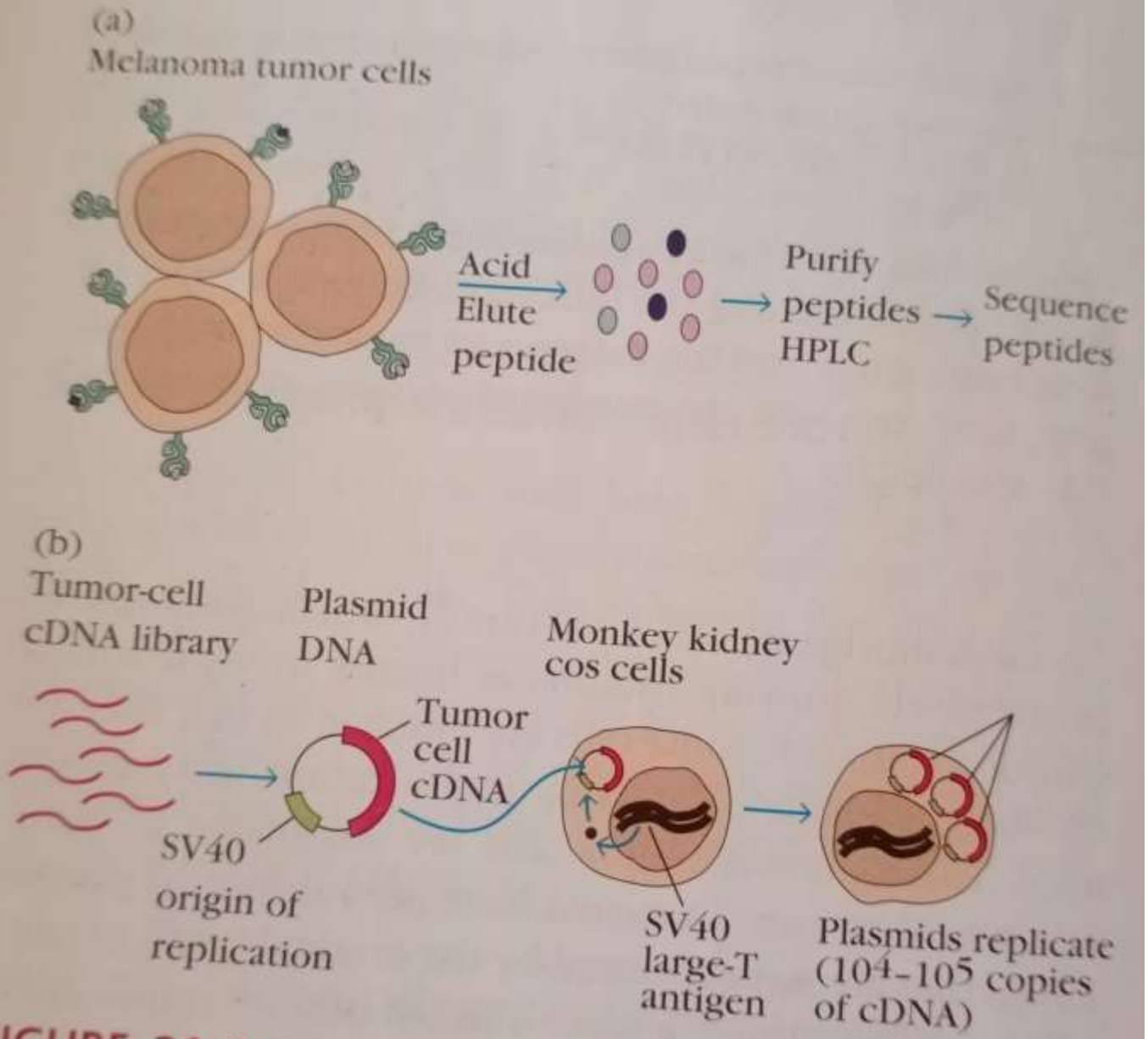


FIGURE 21-8 Two methods used to isolate tumor antigens that induce tumor-specific CTLs. See text for

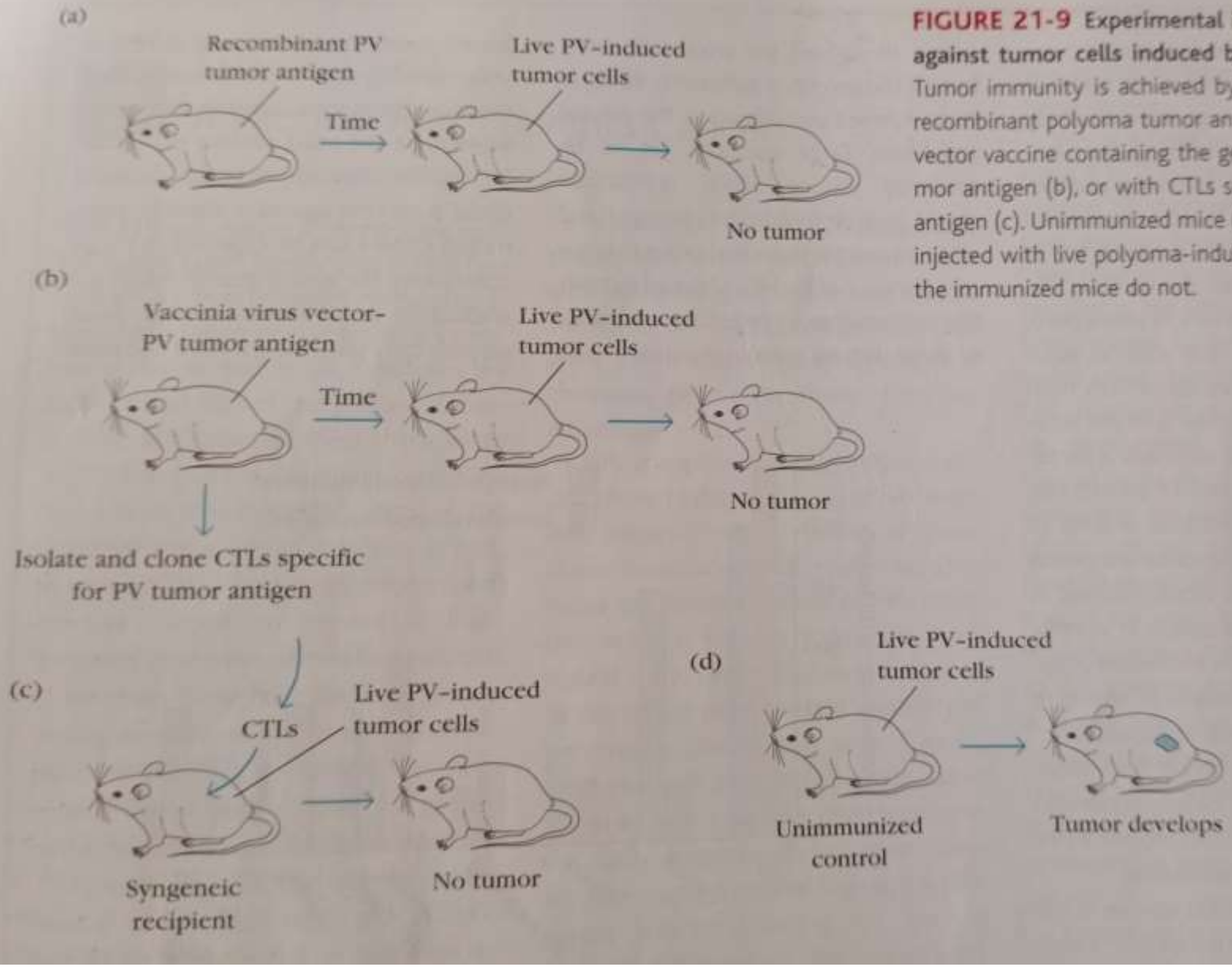


FIGURE 21-9 Experimental induction of immunity against tumor cells induced by polyoma virus (PV). Tumor immunity is achieved by immunizing mice with recombinant polyoma tumor antigen (a), with a vaccinia vector vaccine containing the gene encoding the PV tumor antigen (b), or with CTLs specific for the PV tumor antigen (c). Unimmunized mice (d) develop tumors when injected with live polyoma-induced tumor cells, whereas the immunized mice do not.

References:

- Immunology by Kuby 8th edition
- Basic Immunology 6th edition
- Roitt's Essential Immunology 11th edition
- Textbook of Microbiology- R.Ananthnarayan