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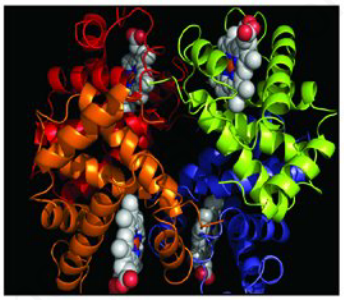
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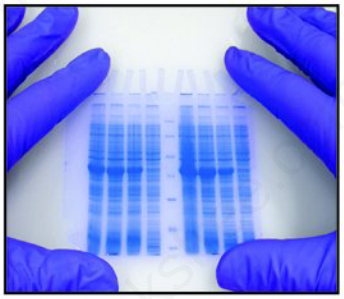
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SELF ASSESSMENT AND REVIEW OF

BIOCHEMISTRY

Second Edition

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Self Assessment and Review of Biochemistry

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Second Edition: **Digital Version 2017**

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Dedication

This book is dedicated to my son, my husband, my parents and in laws, my brothers and to all the students of my classes and facebook group, and the students who could not attend my classes and inspired me to write a book.

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Foreword

Thrissur Medical College Alumni Association (TMCAA) is a CME Programme started in the year 2000 for Junior Doctors guiding and coaching for various PG entrance exams. Our principle is professionalism and structured orientation. Beyond guiding thousands of students to their dream PG and being one of the top institutes in the field we have also generated and promoted many deserving faculties and authors since the inception. Dr Rebecca had started her career as a PG guidance faculty from this institute four years back. Her teaching methodology and updated notes and techniques are well appreciated by students and her notes are of much demand as a "must for exam points". We welcome and appreciate her effort in compiling her notes into students friendly entrance guide. Wishing all success to her and students reading second edition, Self Assessment and Review of Biochemistry.

Ravindran Chirukandathu
MBBS MS (Surgery) DNB FRCS
Chief Coordinator and Secretary
CME for Junior Doctors
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Thrissur, Kerala, India

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President and Coordinator
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Alumni Association (TMCAA)
Thrissur, Kerala, India

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Foreword

It gives me immense pleasure to welcome the second edition of the book "Self Assessment and Review of Biochemistry" written by Dr Rebecca James. She is one of the best faculties in her subject in Calicut Medical College Alumni CME entrance guidance programme. The book comprises a vast collection of questions from the recent exams and thoroughly described. I am sure that now this book will become a necessity among the PG entrance Aspirant's books. Wishing all the success for the book.

NC Cherian MBBS MD (Paediatrics)
Additional Professor of Paediatrics and
Coordinator, Calicut Medical College
Alumni, Medical updates programme
Government Medical College
Calicut, Kerala, India

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Foreword

The second edition of the book "Self Assessment and Review of Biochemistry" has been written as a course material for the various national level (NBE pattern), central institutes, state level, and private medical entrance exams. Dr Rebecca James has been a very keen and devoted teacher in her career. Her new and innovative visual and easy learning techniques have been much useful for her students. The references given are up-to-date and authentic and the presentation and charts are much students friendly. I do recommend this book for PG entrance aspirants. I heartily congratulate her and wish her all the success for this excellent piece of work.

MG Jose Raj MD (Biochemistry)
Professor and HOD, Biochemistry
Government Medical College
Kozhikode, Kerala, India

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Preface to Second Edition

"To improve is to change, to be perfect is to change often ….." **Winston Churchill**

After the overwhelming reception of the first edition of the book across India I am more than excited to release of the second edition. The responses from toppers of various PGMEE speak for the success of the first edition. In this edition we have incorporated the reviews and suggestions about the first edition from readers, yet the same format have been kept in order to remain it as a perfect book for PG aspirants.

Rebecca James MD (Biochemistry)
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Preface to First Edition

Writing a book for Postgraduate Medical Entrance in Biochemistry was a dream for me when I was doing my PG career. But when I started Postgraduate medical entrance coaching, gradually I recognised how hard it was for students to learn the subject. There is no subject expert written book in biochemistry for the major exams like PGI, AIIMS, AIPGME, JIPMER, or even state entrance exams. None of the students had learned biochemistry properly or were not taught properly in their MBBS course and for entrance. Students were forced for learning from books not by a subject expert which are just to pick some points from standard books without explanation or internet reference. Biochemistry comprises up to 8% in NBE exams and up to 30% in PGI exams and lots of researches and updates are coming up in this field. My students often would come asking with doubts quoting some of these guides in which many answers I found wrong. As four years passed in postgraduate medical entrance coaching I made my own notes based on standard textbooks with clinical scenarios and emphasised on teaching to think and correlate rather than mugging up biochemistry. Gradually students from other institutes started asking for my notes and requested me to write a book so that they could also read it. This book is meant for it. I approached Jaypee Brothers Medical Publishers (P) Ltd., and they accepted my proposal, the remaining is in front of you.

This is a PGMEE book and do not compare it to any standard books, but you will get up to 96% if you learn this properly. Biochemistry is a very vast subject with future of research, genetics, which will be overtaking the future of the medical field in time. This book is just a small flower in a big garden. I know that learning in my class would be better than reading this book but due to practical difficulties it is not possible, I have done much justice in including my own made pictures and mnemonics to make it as understandable as possible. I wish that you thoroughly go through this book and gain maximum knowledge from it as it has all the points for scoring top rank in major exams. Wishing you best of luck for your dream PG in the coming exams. Genuine doubts are always welcome. Students can feel free to contact me at gmail/whatsapp. drrebeccajomy@gmail.com

Rebecca James

mebooksfree.com

Acknowledgements

Writing this book has never been easy to me. "***No tears in the writer, no tears in the reader. No surprise in the writer, no surprise in the reader***." — **Robert Frost**.

First and foremost I would like to thank God for enabling me in completing this work. In the process of putting this book together I realized how true the gift of writing is in me. You have given me the power to believe in my passion and pursue my dreams. This would not have been possible without the faith I have in you, the Almighty.

To my husband Dr Jomy, you have been the greatest inspiration and pillar of strength throughout the work. Without your relentless support, this book would have never been materialized.

To my son John, you are the best thing that ever happened to me in my life! This book would have never been complete without you letting me do. Thank you so much.

To my beloved Father Mr. James P George, I am speechless! I can barely find the words to express all the wisdom, love and support you have given me. I am forever grateful for being born to and raised by such an amazing person. If I am blessed to live long enough, I hope I will be a good a parent as you are and always been to me. I love you too, Amma. I also extend my gratitude to my brothers, Mr George James and Mr Philip James.

To my in laws Mrs and Mr Thomas P John, thank you for all the support as my parents and for encouraging me.

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Special thanks to my colleague, Dr Shibu TS, Assistant Professor, Department of Biochemistry, Medical College, Thrissur for his guidance and mental support.

I thank the colleagues of my department (Dr Geetha PA, Dr Jayaraj K, Dr MohamedAshraf, Dr Asha E, Dr Shaji Sreedhar) for their expert opinions and valuable suggestions.

I thank all Senior residents (Dr Anjana, Dr Rejitha Reghunath) and Junior Residents (Dr Anju V, Dr Shajna, Dr Ashuthosh, Dr Sreevalsan, Dr Nasid, Dr Fesina, Dr Rashid) in Department of Biochemistry, Government Medical College, Calicut for your helping hands in arranging the previous questions of various entrance exams and also in the tedious job of proofreading.

I thankfully remember Ms Pradheera M who helped me a lot in typing works.

Also Thanking

- Dr MG Jose Raj, Professor and HOD, Government Medical College, Kozhikode
- Dr Tajan Jose, Coordinator and President TMCAA, Thrissur
- Dr Ravindran Chirukandath, Coordinator and Secretary, TMCAA, Thrissur
- Dr NC Cherian, Calicut Medical College, Weekly Medical Updates, Coordinator
- Dr Arun Kumar, ADR Plexus-Digital strides Coordinator
- Dr Arun Kumar, AIIMS Academic Director
- Dr Manorajan Pozitive, PG Coordinator

Staff and Residents of Department of Biochemistry, Government Medical College, Kozhikode
Dr Akhil K, Dr Goutham Nallaiyan for their mental support
Mr Praveen TMCAA staff

All my students who supported and contributed questions of various PGMEE. Their immense support for making the second edition of the book is impressive. I thank all those who had been on my side on this journey.

HOW TO LEARN BIOCHEMISTRY

Dear students,

I consider you as one of my students by using this book and I believe it is my responsibility to guide you through in your postgraduate medical exams and I wish you all the best to come out with flying colors. I know that many of you feel that Biochemistry is one of the most boring subjects you have learned in MBBS. This is primarily due to the methodology you have been taught and you tend to think of it as a subject of cycles and test tubes. But the fact is that if it was taught with all clinical correlations and the practical applications the subject becomes interesting and beautiful. I hope you will have a renewed perception about biochemistry after reading this book.

This is a concept-oriented book where you learn biochemistry and its applications This is made in a review book style to enable you to answer questions with ease. Another aspect of it is the clinical approach which makes it easier to learn, understand and recollect. Previous questions from all the major exams (PGI, AIIMS, AIPGMEE, STATE ENTRANCE, FMGE, PRIVATE EXAMS and NATIONAL BOARD) are included for discussion.

In learning biochemistry it is recommended to finish the topic you started before moving to another subject so that you can correlate all portions together. You can finish reading the whole book in less than ten days for the first study, five days for revision after three months and two to three days for revision just before your exam. I assure you 96% score. After a careful analysis I find that 40% of questions are direct and another 40% are twisted and a 20% of them are from recent updates. 30 of 200 PGI exam questions are from biochemistry and molecular genetics. AIIMS exam always introduces 2–3 questions about recent updates and NBE which conducts DNB/PGET comprises of 9% questions from Biochemistry which might include pictorial questions and missing parts of certain reactions and clinical questions. Please make sure that you revise the subject as it is a little volatile. This book is different from the usual guide for postgraduate medical entrance exams since every part of it are referred from standard books.

You may feel free to ask your questions, you can mail me or post in my facebook discussion forum. As always, your feedback is important to us. If you believe you have identified an error in the book, please send an email to drrebeccajomy@gmail.com. If you have general comments or suggestions please drop me a line directly to my email. We are continually striving to meet the needs of all individuals preparing for the entrance exams.

Wishing you all the best

Rebecca James
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For author online support, follow
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Image-Based Questions

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Students' Testimonials

Dr Fen Saj

PGI Nov 2015 3rd rank, JIPMER Nov 2015 6th Rank

"I consider myself fortunate to have attended Dr Rebecca's high yield lectures at TMCAA. She is a very dedicated teacher and makes the core concepts in Biochemistry simple and interesting. Her book, Self assessment and review of Biochemistry is a must read for all entrances especially for PGI as they ask almost 20 + questions from biochemistry and related topics. The book will compensate for those who cannot attend her classes as it is well written, updated, error free and revision friendly."

Dr Ram Manohar Talupula

AIIMS Nov 2015 2nd Rank

"I heard about Dr Rebecca's Self assessment and review of Biochemistry during my PG preparation time, when I was confused about what book to read for Biochemistry. I feel this book as a perfect example of how an entrance preparation book should be. This helped me a lot especially during the last few days before the exam, as it is concise and at the same time high yielding"

Dr Vimal Chacko

JIPMER Nov 2015 Rank 6, AIIMS Nov 2015 Rank 53

"Madam, I am your student in TMCAA. I want to thank you so much. All the questions that came from Biochemistry was covered by you. Your classes made the difference."

Dr Nabeel Faizal

AIIMS Rank 193

"Thank you for your valuable teaching and guidance."

Dr Mohammed Ameen

AIIMS Nov 2015 Rank 13

"Thanks a lot for your classes and wonderful book. I could answer many questions from Biochemistry. Madam's class and book matter a lot to me."

Dr Aishwarya

DNB Dec 2015 Rank 25

"Thank you mam…Your classes and book have always given us the extra edge. Whatever we know about Biochemistry is only because of you."

Dr Akhil K Aravind

AIIMS Rank 92

"Thank you so much mam. All biochemistry questions were from your class and book only. I attended all biochemistry questions with confidence. That is the success of a good teacher like you mam. God bless u"

Dr Uma Shankar

"Mam got 250 rank in AIIMS. Your page and information provided by you helped me a lot"

Dr Arjun

"I got 27th rank in JIPMER Nov 2015. Your classes were excellent and inspiring and everything you taught only was asked. Your last minute revision in the facebook group was really useful. Thank you so much mam"

PG Aspirant from Thirunelveli

"It is written in my fate that I should understand the true core of biochemistry after MBBS through your book. In Islam it is told that to sustain your life even after death is to give charity and to teach the ignorant. Mam you are one who deserves that place. Hats off to you mam to give us knowledge of Biochemistry through your book"

Dr Nikhil

" Hello mam this is Nikhil from Banglore. I am preparing for PG entrance, I bought your book. I usually hate biochemistry, but after reading the first chapter itself I fell in love with biochemistry. It is an awesome way of explanation you have given mam"

Dr Sindhu Jonnala

" Thank you mam for such a wonderful book. You have just simplified everything. Mentioned all must know topics. The high-yielding points represented very well. I was always having fear for Biochem, it was a nightmare for me. Your book boost up my confidence for preparation. Thank you mam for such big help"

Dr Payal Kumari

"Thank you mam for your book and forum in facebook. I attempted all biochem questions correctly in AIPGMEE and DNB"

Dr Swati Dash

"Biochem was the dreadful subject in my career. I am very poor in basics. Then I got to know about your book. I can not express my gratitude and words enough to express the amount of confidence your notes in the book have instilled in me now. Thank you for the wonderful book"

Dr Nilotpal Bhattacherjee

"Mam exam was good. All from your book and updates in the facebook group"

Dr Deepika Mishra

"Hello mam. Someone suggest your book last year and may I just say that its brilliant. I have struggled with biochemistry, the book made so many concepts clear to me, even everything asked in PGI and AIIMs this year was mentioned in your book. So thanks a lot."

Dr Surbhi Jain

"Thank you mam for this time in AIIMS biochemistry questions I was more confident in answering because I could recall the questions as direct lines from your book"

Dr Sharath M Adiga

"Hi mam I would like to thank you for your guidance in TMCAA and your awesome book. I got rank 158 in AIIMS"

Dr Saravanan Ganesan

"Thank you for your wonderful lecture and book I got rank 78 in AIIMS in UR category"

Dr Vigneshwar Sivaraman

All questions in DNB were direct from your book. Your lecture is the best of biochem I ever attended"

Dr Navin Srinivasan

"Mam your book is too good. I like biochemistry and Harper a lot. Your book is no less than Harper "

Dr Naveen Murali

"I read your book though not fully, but I attended all biochem question in Nov AIIMS 2015. I always felt I would not read biochem, but after buying your book things changed. Thanks a lot"

Dr Aqhib Nadeem

"Mam I am your student in TMCAA. I am extremely to your ultimate teaching and your book. I could read whole biochemistry in just 2 and half days, and answer almost all the biochemistry questions correctly"

Dr Ragesh Ravindran

"All questions from your book mam. Me and Calicut Medical College is proud of you"

Dr Neha Thakur

"Your book is an epic mam. I marked almost all questions right. I am happy because for the first time I could solve biochemistry"

Dr Vimmi Gautam

"You are the best mam. Your words were questions. Mam biochem I felt so happy to answer because of you"

Dr Prachi Gupta

"Biochemistry was a dreaded subject for me. And in my previous attempt I did not even touch this subject as it looked scary. A friend of mine referred me your book. I bought it and read it. I must say "What an effort mam!!! I am so happy and satisfied with your book and it gave me confidence that 'Yes, even I can attempt biochemistry' questions."

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1

SECTION | Amino Acids and Proteins

CHAPTERS

1. Chemistry and Metabolism of Amino Acids
2. Proteins
3. Enzymes

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1 Chemistry and Metabolism of Amino Acids

Topics Included

- **Chemistry of Amino Acids:**
  - Classification of Amino Acids
  - Properties of Amino Acids
- **Metabolism of Amino Acids:**
  - General Amino Acid Metabolism
  - Individual Amino Acid Metabolism

CHEMISTRY OF AMINO ACIDS

General Structure of Alpha Amino Acid

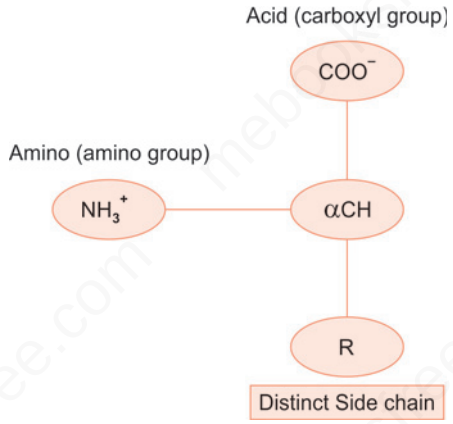


Fig. 1.1: General structure of alpha amino acid

Alpha Amino Acid

- Amino group and carboxyl group attached to the alpha carbon atom
- Most of the amino acids are alpha amino acid.

Non Alpha Amino Acid

Unlike alpha amino acids either carboxyl group or amino group is not attached to the alpha carbon atom.

Non alpha amino acids present in tissues in free form are:

- β Alanine
- β Aminoisobutyrate
- γ –Aminobutyrate

Imino Acid

- In an imino acid amino group is not free
- The nitrogen of amino group is seen inside the Pyrrolidine ring
- Still it can form a peptide bond
- Proline is an imino acid.

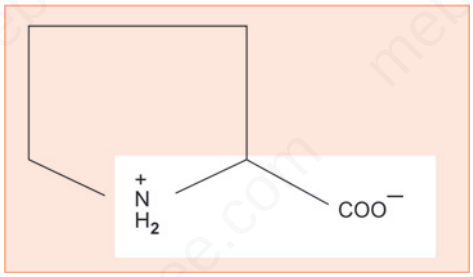


Fig. 1.2: Structure of imino acid, proline

CLASSIFICATION OF AMINO ACIDS (VERY IMPORTANT TOPIC)

- Based on the variable side chain (R group)
- Based on side chain characteristics (Polarity)
- Based on nutritional requirement
- Based on metabolic fate.

BASED ON VARIABLE SIDE CHAIN[Q]

Aliphatic Amino Acid

- **Simple Amino Acid**
  - Glycine[Q]
  - Alanine

- **Branched Chain Amino Acid**[Q]
  - **L**eucine
  - **I**soleucine
  - **V**aline

Mnemonic for Branched Chain Amino Acid -LIV Amino Acid

- **Sulfur Containing Amino Acid**
  - Cysteine[Q]
  - Methionine[Q]
- **Amino Acid with Hydroxyl Group**
  - Serine[Q]
  - Threonine[Q]
- **Amino Acid with Amide Group**
  - Asparagine
  - Glutamine
- **Acidic Amino Acid**
  - Aspartic Acid (Aspartate)
  - Glutamic Acid (Glutamate)
- **Basic Amino Acid**
  - Arginine (Most Basic Amino acid)[Q]
  - Lysine

Aromatic Amino Acid

- Phenylalanine[Q]
- Tyrosine[Q]

With heterocyclic aromatic ring (Ring structure contain more than one type of atom.)

- Tryptophan[Q]
- Histidine (Basic Amino Acid)[Q].

- Aromatic amino acid with hydroxyl group is Tyrosine
- Aromatic amino acid with basic properties is Histidine.

Imino Acid

- Proline

BASED ON SIDE CHAIN CHARACTERISTIC (POLARITY)[Q]

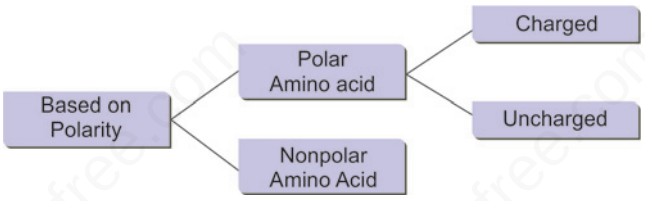


Fig. 1.3: Classification of amino acid based on polarity

- **Polar Amino Acids**[Q] **(Hydrophilic)**
  - Charged
    - Acidic Amino acids-Aspartic Acid (Aspartate), Glutamic Acid (Glutamate)
    - Basic Amino Acids-Histidine, Arginine, Lysine
  - Uncharged
    - Aliphatic amino acid with hydroxyl group as side chain: Serine, Threonine
    - Aliphatic amino acids with amide group: Asparagine, Glutamine
    - Simple Amino acid: Glycine alone
    - Sulfur containing Amino acid: Cysteine alone.
- **Nonpolar Amino Acid**[Q] **(Hydrophobic)**
  - Simple amino acid: Alanine alone
  - Sulfur containing amino acid: Methionine alone
  - Aromatic Amino acids except Histidine *(Think as it is basic amino acid it is already included among polar amino acid)*
  - All branched chain amino acids: Leucine, Isoleucine and Valine
  - Imino acid: Proline

Concept

- Learn polar and nonpolar amino acid by classifying the amino acid rather than learning individual amino acid
- Charged amino acids are polar. Charged amino acids are Acidic and basic amino acids
- Mnemonic ABC: **A**cidic and **B**asic amino acids are Charged amino acids
- All branched chain amino acids are nonpolar
- All aromatic amino acids except Histidine are nonpolar.

BASED ON METABOLIC FATE[QQQ]

Classified into:

- *Ketogenic:* Amino acids that are converted to Acetyl CoA and thereby to Ketogenic Pathway
- *Glucogenic:* Amino Acids that enter into Glucogenic pathway
- *Both Glucogenic and Ketogenic:* That can enter into both ketogenic and glucogenic pathway.

Classification of amino	Amino acid
Purely Ketogenic	Leucine[Q]
Both Ketogenic and Glucogenic	**P**henylalanine
	Isoleucine
	Tyrosine
	Tryptophan

Contd...

Contd...

Classification of amino	Amino acid
	Lysine* (Predominantly Ketogenic)
Glucogenic	Any amino acid that do not belong to the above groups

Concept

First learn the amino acids which are Ketogenic, and then learn amino acids which are both Glucogenic and Ketogenic

Mnemonic to learn both Ketogenic and Glucogenic-LPITT (Lysine, Phenylalanine, Isoleucine, Tyrosine, Tryptophan)

BASED ON NUTRITIONAL REQUIREMENT[QQQ]

- *Essential:* Those amino acids which cannot be synthesised in the body[Q]. Hence these amino acids are to be supplied in the diet.
- *Semiessential:* Growing children require them in the food, but not essential in adults.
- *Nonessential:* Amino acids which can be synthesised in the body[Q], hence not required in the diet.

Essential	Semiessential	Nonessential
Methionine	Arginine	All the other amino acids
Threonine		
Tryptophan		
Valine		
Isoleucine		
Leucine		
Phenylalanine		
Lysine		
Histidine		

- Mnemonic to learn essential amino acids-MeTT VIL PHLY (read as Met will fly). Methionine,Threonine, Tryptophan,Valine, Isoleucine, Leucine, Phenylalanine, Lysine

Special Groups Present in Amino Acids

Amino acid	Special group
Arginine	Guanidinium[Q]
Phenylalanine	Benzene
Tyrosine	Phenol
Histidine	Imidazole[Q]
Proline	Pyrrolidine
Methionine	Thioether linkage
Tryptophan	Indole
Cysteine	Thioalcohol (SH)

Conservative (Homologous) Substitution

One amino acid replaced by another amino acid of similar characteristics

Examples of homologous substitution is shown in the diagram given below.

Conservative Mutation

Hydrophilic, Acid	Asp	Glu				
Hydrophilic, Basic	His	Arg	Lys			
Polar, Uncharged	Ser	Thr	Gln	Asn		
Hydrophobic	Ala	Phe	Leu	Ile	Val	Pro

Nonconservative (Nonhomologous) Substitution

One amino acid replaced by another amino acid of different characteristics.

ABBREVIATIONS OF AMINO ACIDS

Amino acids with unique first letter

Amino acid	Three letter Abbreviation	One letter Abbreviation
Cysteine	Cys	C
Histidine	His	H
Isoleucine	Ile	I
Methionine	Met	M
Serine	Ser	S
Valine	Val	V

Amino acids which do not have unique first letter

Abbreviated based on the commonly occurring amino acids		
Amino acid	**Three letter Abbreviation**	**One letter Abbreviation**
Glycine	**Gly**	**G**
Alanine	**Ala**	**A**
Leucine	**Leu**	**L**
Proline	**Pro**	**P**
Threonine	**Thr**	**T**

Abbreviated based on phonetically sounding letters		
Amino acid	**Three letter Abbreviation**	**One letter Abbreviation**
Arginine	**Arg**	**R (aRginine)**
Asparagine	**Asn**	**N (asparagiNe)**
Aspartatic Acid	**Asp**	**D (asparDic acid)**
Glutamic Acid	**Glu**	**E (glutEmic acid)**
Glutamine	**Gln**	**Q (Qtamine)**
Phenylalanine	**Phe**	**F (Fenylalanine)**

Contd...

Contd...

Abbreviated based on phonetically sounding letters		
Amino acid	**Three letter Abbreviation**	**One letter Abbreviation**
Tyrosine	**Tyr**	**Y (tYrosine)**
Tryptophan	**Trp**	**W (tWiptophan)**
Abbreviated based on letter close to initial letter		
Lysine	**Lys**	**K (letter close to L)**

21st and 22nd Amino Acids[Q]

Selenocysteine

21st Protein forming Amino Acid[Q]

Precursor amino acid for selenocysteine is Serine[Q]

Serine is modified to cysteine.

Selenium replaces sulfur of cysteine cotranslationally

In humans approximately 2 dozen selenoproteins are there, that includes Peroxidase[Q] and Reductases[Q].

Seen in the active site of following Enzymes and Proteins[Q]

- Thioredoxin reductase
- Glutathione peroxidase
- Iodothyronine deiodinase
- Selenoprotein P

Coded by the Stop Codon, **UGA[Q]** by a process called **Recoding**

SECIS Element in the mRNA helps in this process

Pyrrolysine

- 22nd protein forming Amino Acid
- By recoding **UAG** stop codon, helped by PYLIS element in the mRNA.

DERIVED AMINO ACIDS

Derived Amino Acid seen in Protein[Q]

4-Hydroxyproline	• Found in Collagen • Vitamin C is needed for hydroxylation.
5-Hydroxylysine	
Methyllysine	Found in Myosin
Gammacarboxy glutamate	• Found in clotting factors, like Prothrombin that bind Ca^{2+} • Vitamin K is needed for Gammacarboxylation.
Cystine	• Found in proteins with disulphide bond.[Q 2014 DNB] • Two cysteine molecules join to form cystine, e.g. Insulin, Immunoglobulin
Desmosine	• Found in Elastin[Q AIIMS Nov 2014]

Derived Amino Acid not seen in Protein[Q]

Ornithine	Intermediates of Urea Cycle
Arginosuccinate	
Citrulline	
Homocysteine	Derived from Methionine[Q]
Homoserine	Product of Cysteine Biosynthesis
Glutamate-γ Semialdehyde	Serine Catabolite

RECENT UPDATES

Extraterrestrial Amino Acids

In february 2013 following explosion of approximately 20,000 metric ton meteor in the skies in chelynabinsk, west siberia extra terrestrial amino acids like alanine, aspartic acid, glutamic acid, isoleucine, leucine, phenylalanine, serine, threonine, tyrosine, valine, n methyl glycine, β alanine were found in the remnants of meteors. These findings demonstrated potential insights to existence of extraterrestrial life.

PROPERTIES OF AMINO ACID

A Genetic Code Specifies an Amino Acid

More than 300 naturally occurring amino acids exist in nature out of which 20 amino acids constitute monomer units of proteins.

Remember

- Amino acids not coded by genetic code are all the derived amino acid.[Q]
- Amino acids coded by stop codon are Selenocysteine, Pyrrolysine

Amino Acid Exists in Three Charged State, Positive, Negative or Neutral

Depends on the two factors:

1. Isoelectric pH of the amino acid.
2. pH of the surrounding medium.

Isoelectric pH of Amino Acids

- **At pH = Isoelectric pH**
- **At pH < Isoelectric pH**
- **At pH > Isoelectric pH**

At pH = Isoelectric pH (pI)

- The amino acid carry equal number of positive and negative charge, i.e. **NO NET CHARGE.**
- Amino acid exist as **ZWITTER ION (AMPHOLYTE)**

Zwitter Ions or Ampholytes

Molecules which carry equal number of ionizable groups of opposite charge and therefore bear no net charge are called **Zwitter ions or ampholytes[Q].** Zwitter is a german word which means hermaphrodite.

Properties of Amino acid at Isoelectric pH(pI)

- No mobility in electric field[Q]
- Minimum solubility
- Maximum precipitability[Q]
- Minimum buffering capacity

At pH less than isoelectric pH (pI)

- Amino acid exists as protonated or positively charged.

At pH greater than isoelectric pH (pI)

- Amino acid exists as deprotonated or negatively charged.

The charge of carboxyl group and amino group at physiological pH (pH = 7.4)

- Carboxyl group is negatively charged
- Amino group is positively charged.

Amino Acids Exhibit Isomerism

Amino acids have asymmetric (chiral) alpha carbon atom. The mirror images produced with reference to alpha carbon atom, are called D and L forms or enantiomers.

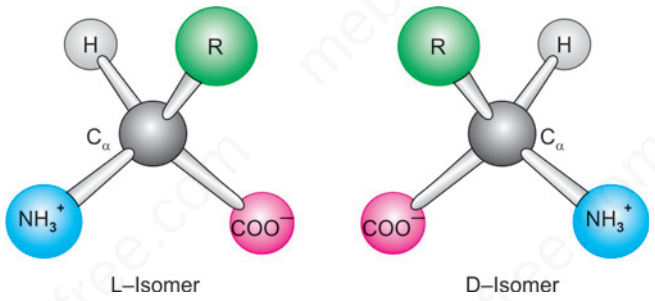


Fig. 1.4: L and D amino acid

- Almost all naturally occurring Amino Acids are **L-Isomers**
- Some naturally occurring Amino acids are D Amino acids.

Naturally occurring D Amino Acid

- Free D Aspartate and Free D Serine in brain tissue
- D-Alanine and D Glutamate in cell walls of gram-positive bacteria
- *Bacillus subtilis* excretes D-methionine, D-tyrosine, D-leucine, and D-tryptophan to trigger biofilm disassembly
- *Vibrio cholerae* incorporates D-leucine and D-methionine into the peptide component of their peptidoglycan layer.

Remember

Amino Acid with No Chiral/No Asymmetric/No Optically Active Carbon **Glycine**[QQQ]

Source of D-Amino acids in humans is exogenous.

Potentially Toxic L-Amino Acids

- Certain L α Amino acids present in the in plants can adversely affect human health
- Present in the seeds of certain species of Lathyrus.

Examples of Toxic L-Amino Acids

L- Amino acids	Clinical implication
L-Homoarginine cleaved by Arginase to L Lysine and Urea	Causes Neurolathyrism in humans
β N Oxalyl Diamino Propionic Acid (β ODAP)	Neurotoxin Causes Neurolathyrism in humans
β N Glutamyl Amino Propiono Nitrile (BAPN)	An Osteolathyrogen
2, 4-Diaminobutyric acid	Inhibits ornithine transcarbamylase, resulting in ammonia toxicity.
β-Methyl amino alanine (Present in Cycad seeds)	Possible risk factor for neurodegenerative diseases like • Parkinson's Disease • Amyotropic Lateral Sclerosis (ALS)

Amino Acid Absorb UV Light[Q]

Amino Acids which absorb **250–290 nm** (Maximum at **280 nm**) UV light are **tryptophan, phenylalanine, tyrosine.** Maximum absorption of UV light by **tryptophan.**[Q]

Remember

- Aromatic amino acids absorb UV light.
- Amino acids are colorless because they do not absorb visible light.

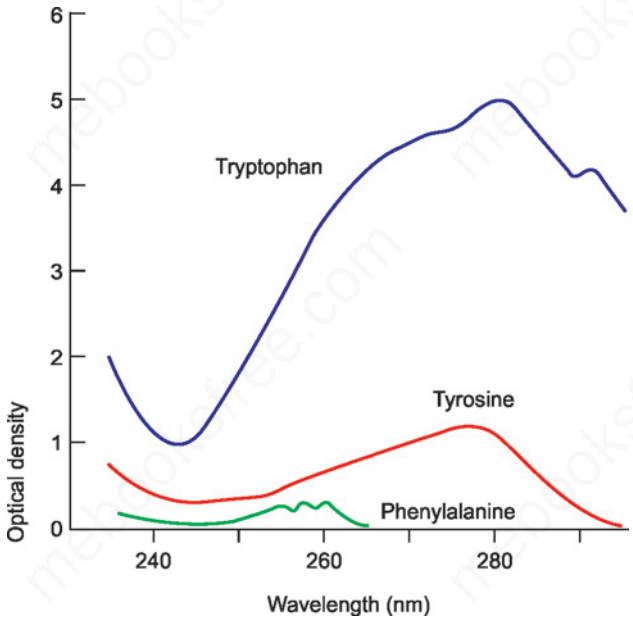


Fig. 1.5: Ultraviolet absorption spectra of aromatic amino acids

BETA–ALANINE (Very important topic for national board pattern exams)

- Formed from Cytosine and Uracil[QDNB/AIPGMEE]
- Other sources of Beta Alanine is hydrolysis of Beta alanyldipeptides

- Beta Alanine is seen in[Q DNB/AIPGMEE]
  - Pantothenic Acid
  - Coenzyme A
  - Acyl Carrier Protein
  - Beta Alanyl Dipeptides.
- **Beta Alanyl Dipeptides are:**
  - Carnosine [Histidine + Beta alanine]
  - Anserine [N methyl Carnosine] Both present in **Skeletal muscle.**

Uses of Carnosine

- Activate Myosin ATPase
- Chelate Copper
- Enhance Copper uptake
- Buffers the pH of anaerobically contracting muscle.

Remember
- Homocarnosine is GABA + Histidine
- There is no beta alanine in homocarnosine

DECARBOXYLATION OF AMINO ACID

- The amino acid undergo alpha decarboxylation to form corresponding Amines
- **PLP[Q] is the coenzyme for this reaction.**

Examples of Amino Acid Decarboxylation

Amino acid	Biologic amines
Histidine	Histamine
Tyrosine	Tyramine
Tryptophan	Tryptamine
Lysine	Cadaverine
Glutamic Acid[Q]	Gamma Amino Butyric Acid (GABA)
Serine	Ethanolamine
Cysteine	Beta mercaptoethanol amine

Points to Ponder
- Amino acids undergo decarboxylation to form corresponding amines. Most of them are biologically important hence called Biologic Amines.
- PLP is the coenzyme for Amino Acid Decarboxylation
- Amino acids undergo deamination to form corresponding Ketoacids.
- Most common amino acid that undergo Oxidative deamination is Glutamic Acid (Glutamate)
- Glutamic acid undergo decarboxylation to form GABA
- Glutamic Acid undergo deamination to form Alpha Ketoglutarate

COLOR REACTIONS OF AMINO ACIDS

Biuret Test

- General test for proteins
- Cupric ions in alkaline medium forms violet color with peptide bond nitrogen.

Remember
Dipeptides and individual amino acid do not answer biuret test because this test needs a minimum of two peptide bonds.

Ninhydrin Test

General test for all alpha Amino Acid
Amino acid + 2 mols of Ninhydrin -------> Aldehyde with 1 carbon atom less + CO_2 + Purple Complex (Ruhemann's Purple)

Remember
- **Amino acid which do not give purple color are:**
- Proline and Hydroxy proline (Yellow color)
- Glutamine and Asparagine (Brown color)

Colour Reactions	Test answered by
Xanthoproteic Test **(Conc HNO_3 is a reagent[°])**	**Aromatic Amino Acid**[2014 DNB] (Phenylalanine, Tyrosine, Tryptophan)
Millon's test	Tyrosine (Phenol)
Aldehyde test can be done in two methods: • Acree Rosenheim Test (Formaldehyde and Mercuric Sulfate is used) • **Hopkin's Cole Test[Q]** • (Glyoxylic Acid is used)	Tryptophan (Indole group)
Saka **G**uchi's test	Ar**G**inine (**G**uanidinium group)

	Mnemonic–G is common to all
Sulfur test	Cysteine
Cyanide Nitroprusside Test	Homocysteine
Pauly's Test	Histidine (Imidazole) Tyrosine (Phenol)

Methionine does not answer Sulfur test because sulfur in methionine is in the thioether linkage which is difficult to break.

Buffering Action of Amino Acids

- Buffers are solutions which can resist changes when acid or alkali is added.

Henderson Hasselbalch Equation
pH = pKa + log [Base]/[Acid]
When [Base] = [Acid] pH = pKa

Maximum buffering capacity is at **pH = pKa**. So amino acid which has pKa range near physiologic pH can act as an effective buffer.

pKa Range of Amino Acid

Dissociating group	pKa range
Alpha carboxyl group	3.2–4.1
Non alpha COOH of Asp and Glu	4.0–4.8

Contd...

Contd...

Dissociating group	pKa range
Imidazole group of histidine	6.5–7.4
SH group of Cysteine	8.5–9.0
OH group of Tyrosine	9.5–10.5
Alpha amino group	8.0–9.0
Guanidinium group of Arginine	> 12

At physiologic pH Imidazole group of Histidine has the maximum buffering capacity[Q]

High-yielding Facts-Amino Acids

- Simplest Amino acid-Glycine
- Most hydrophobic (nonpolar) Amino acid-Isoleucine
- Second most nonpolar amino acid is Valine
- Most Polar amino acid is Arginine
- Most abundant amino acid in the proteins present in the body is Alanine
- Most abundant amino acid in the plasma-Glutamine

Amino Acids and Amino Acid Derivatives as Neurotransmitters

- Glycine-Major inhibitory neurotransmitter in brain stem and spinal cord
- Glutamate-Major excitatory neurotransmitter.

Amino Acid Derivative as neurotransmitter

- Dopamine
- Epinephrine
- Norepinephrine
- Serotonin
- Gamma Amino Butyric Acid (GABA).

DIGESTION OF PROTEINS

Native proteins are resistant to digestion because few peptide bonds are accessible to the proteolytic enzymes without prior denaturation of dietary proteins (by heat in cooking and by the action of gastric acid).

Enzymes Catalyze the Digestion of Proteins

There are two main classes of proteolytic digestive enzymes (proteases)

1. **Endopeptidases** hydrolyze peptide bonds between specific amino acids throughout the molecule. They are the first enzymes to act, yielding a larger number of smaller fragments.
   - **Pepsin** in the gastric juice catalyzes hydrolysis of peptide bonds adjacent to amino acids with bulky side-chains (aromatic and branched-chain amino acids and methionine).
   - **Trypsin, chymotrypsin, and elastase** are secreted into the small intestine by the pancreas.
     - Trypsin catalyzes hydrolysis of lysine and arginine esters.
     - Chymotrypsin catalyzes hydrolysis esters of aromatic amino acids.
     - Elastase catalyzes hydrolysis esters of small neutral aliphatic amino acids.
2. **Exopeptidases** ***catalyze the hydrolysis of peptide bonds, one at a time, from the ends of peptides.***
   - Carboxypeptidases, secreted in the pancreatic juice, release amino acids from the free carboxyl terminal.
   - Aminopeptidases, secreted by the intestinal mucosal cells, release amino acids from the amino terminal.
   - Dipeptidases and tripeptidases in the brush border of intestinal mucosal cells catalyze the hydrolysis of di-and tripeptides, which are not substrates for amino-and **carboxypeptidases.**

The proteases are secreted as inactive **zymogens;** the active site of the enzyme is masked by a small region of the peptide chain that is removed by hydrolysis of a specific peptide bond.

Pepsinogen is activated to pepsin by gastric acid and **by activated pepsin.**

In the small intestine, trypsinogen, the precursor of trypsin, is activated by **enteropeptidase**[Q], which is secreted by the duodenal epithelial cells; trypsin can then activate chymotrypsinogen to chymotrypsin, proelastase to elastase, pro-carboxypeptidase to carboxypeptidase, and pro-aminopeptidase to aminopeptidase.

GENERAL AMINO ACID METABOLISM

Biosynthesis of Urea

Urea biosynthesis occur in four stages:

1. Transamination

Definition

- Transfer of alpha amino group from one amino acid to a keto acid to form another pair of amino acid and keto acid.
- Amino group from amino acids are concentrated in the form of Glutamate.
- Because only **Glutamate can undergo oxidative deamination** to significant amount thereby releasing ammonia that enter into urea cycle.

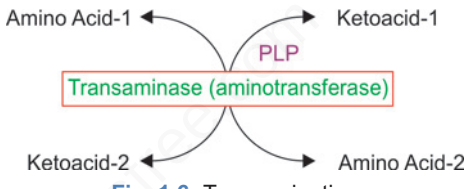


Fig. 1.6: Transamination

Important Points—Transamination

- Transaminase (Amino transferase) is the enzyme
- Pyridoxal Phosphate (PLP) a derivative of Vitamin B6 is the Coenzyme
- Occur in all the tissues
- This reaction occurs via Double Displacement (Ping Pong) mechanism
- No free ammonia is liberated
- Freely reversible
- Play an important role in biosynthesis of nutritionally nonessential amino acid.

Examples of transamination

- **Alanine Amino Transferase (ALT) or Serum Glutamate Pyruvate Transaminase (SGPT)**
  Alanine + α-Ketoglutarate $\rightarrow$ Pyruvate + Glutamate
- **Aspartate Amino Transferase (AST) or Serum Glutamate Oxaloacetate Transaminase (SGOT)**
  Aspartate + α-Ketoglutarate $\rightarrow$ Oxaloacetate + Glutamate

Amino Acid that do not undergo Transamination[QDNB/AIPGMEE]

- Proline
- Hydroxyproline
- Threonine
- Lysine

Delta Ornithine Aminotransferase

- Apart from α amino group of amino acid, δ amino group of Ornithine can undergo transamination.

Clinical Correlation

- Ornithine δ Aminotransferase deficiency can lead to Gyrate Atrophy of Retina and Choroid
- Treatment involve restriction of dietary Arginine
- Pyridoxine is given as a treatment.

2. Oxidative Deamination

- The removal of amino group from amino acid is called deamination
- Only glutamate can undergo significant oxidative deamination.

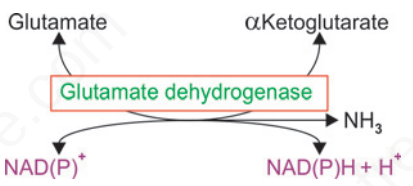


Fig. 1.7: Oxidative deamination

Oxidative Deamination—Important Points

- Glutamate Dehydrogenase (GDH) is the enzyme
- Liver mitochondria contain Glutamate Dehydrogenase (GDH)
- NAD^+ or $NADP^+$ is the coenzyme for the enzyme
- Releases Nitrogen as Ammonia which enter into Urea Cycle
- Reversible
- Liver GDH is allosterically inhibited by ATP, GTP, and $NADH^Q$
- Liver GDH is activated by ADP.

L-Amino acid oxidase

Minor pathway of deamination of amino acids:

- Takes place in the liver and kidney
- FMN is the coenzyme of this reaction
- H_2O_2 is formed[QDNB]

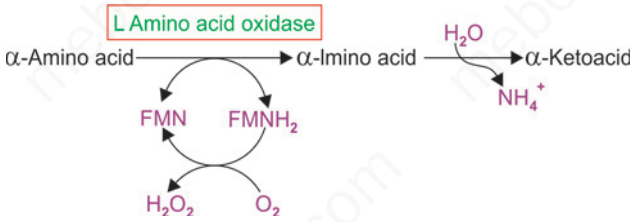


Fig. 1.8: L amino acid oxidase

Some examples of Nonoxidative Deamination^Q NBE pattern

- **Amino acid Dehydrases** for amino acids with hydroxyl group (Serine, Threonine)
- **Histidase** for histidine
- **Amino acid Desulfhydrases** for amino acids with sulfhydryl group, Cysteine and Homocysteine

Transdeamination

Conversion of α Amino nitrogen to ammonia is by concerted action of amino tranferase and Glutamate Dehydrogenase is often termed as Transdeamination.

Transamination + Oxidative Deamination = Transdeamination

3. Transport of Ammonia (Very Important Topic PGMEE Exams)

Free ammonia is toxic to cells especially to brain. Excess ammonia generated has to be converted to nontoxic form Then transported to liver to enter into urea cycle.

- Transport of Ammonia from most of the tissues including the brain
- Transport of Ammonia from skeletal muscle.

Transport of Ammonia from most of the tissues including the brain.

- As **Glutamine**^{Q} with the help of the enzyme Glutamine Synthetase.

Glutamine Synthetase

- Ammonia formed in most tissues including the brain is trapped by Glutamate to form Glutamine
- This is called first line trapping of ammonia
- ATP is required for this reaction.

Glutaminase

- In the liver, glutaminase removes the ammonia from Glutamine.
- Ammonia enter into urea cycle in the liver.

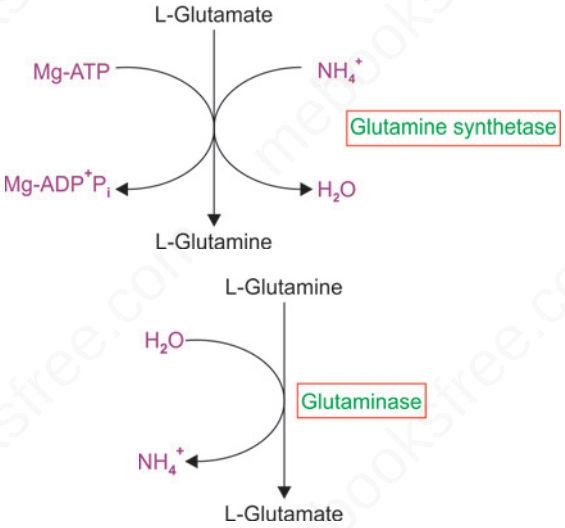


Fig. 1.9: Glutamine synthetase and glutaminase

Transport of Ammonia from skeletal muscle.

- From Skeletal Muscle as **Alanine**Q
- In skeletal muscle, excess amino groups are generally transferred to pyruvate to form alanine.

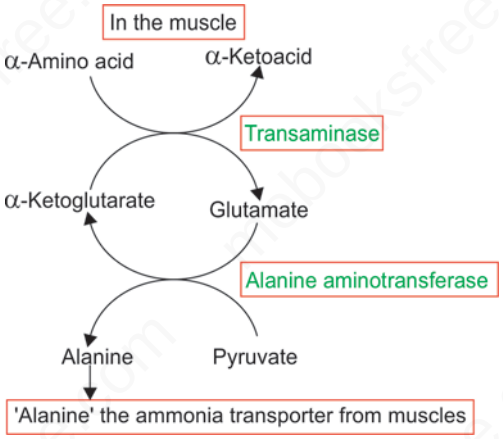


Fig. 1.10A: Transport of ammonia from different organs

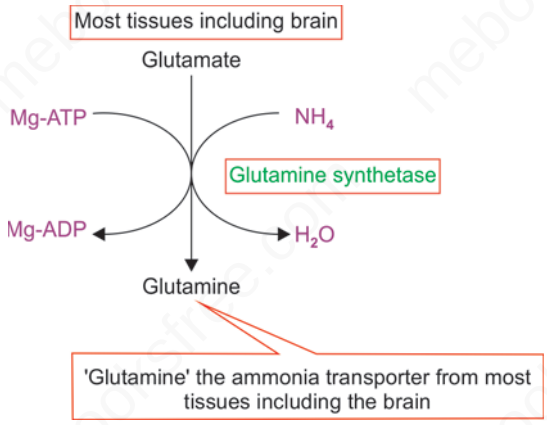


Fig. 1.10B: Transport of ammonia from different organs

4. Disposal of Ammonia

- The ammonia from all over the body reaches the liver. It is then detoxified to urea by liver cells, then excreted through kidney
- UreaQ is the major end product of protein catabolism in the body.

Sources of UreaQ

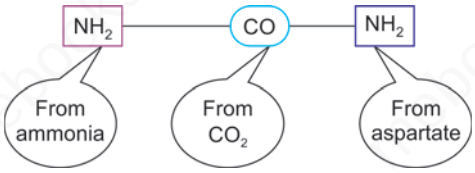


Fig. 1.11: Sources of nitrogen and carbon atoms of urea

UREA CYCLE (VERY IMPORTANT TOPIC)

The urea cycle is the first metabolic pathway to be elucidated by Sir Hans Krebs and a medical student associate, Kurt Henseleit hence as **Krebs Henseleit Cycle**.

- Ornithine consumed in the reaction 2 is regenerated in the reaction 5. Hence called **Ornithine Cycle.**

Site of Urea Cycle

- *Organ:* Takes place in liver.
- *Organelle:* Partly **mitochondrial and partly cytoplasmic**.

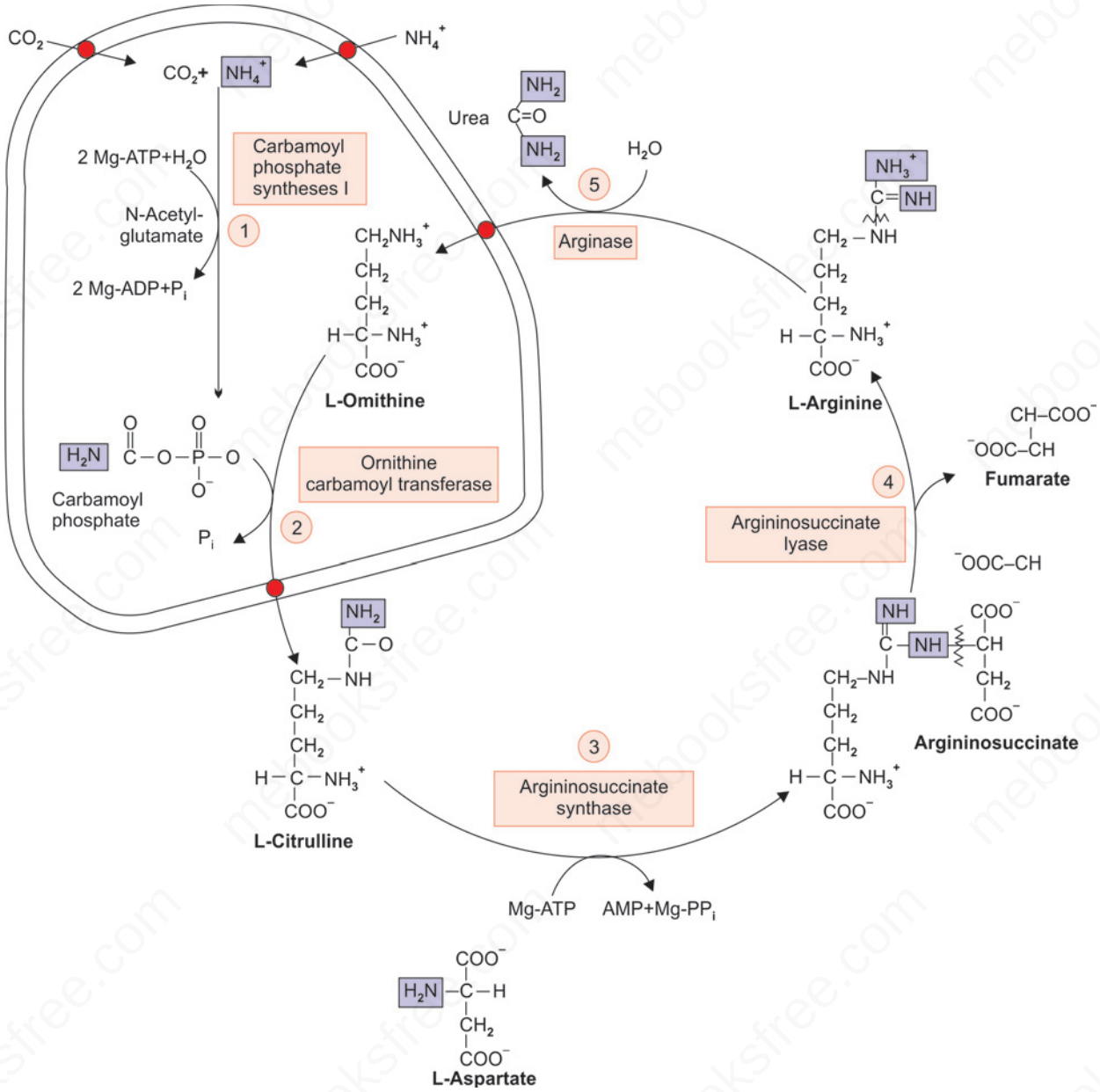


Fig. 1.12: Reactions of urea cycle

Reactions of Urea Cycle

The first two reaction takes place in the mitochondria. The rest of the reactions takes place in the cytoplasm.

Carbamoyl Phosphate Synthetase–I (CPS-I)

- Carbamoyl Phosphate is formed from the condensation of CO_2, Ammonia and ATP
- Takes place in the mitochondria
- CPS-I is the rate limiting (pacemaker) enzyme in this pathway.
- Cytosolic CPS-II is involved in Pyrimidine synthesis
- CPS-I is active only in the presence of N-Acetyl Glutamate, an allosteric activator[Q]
- This step require 2 mols of ATPs.

Ornithine Transcarbamoylase (OTC)

- Transfer carbamoyl group of Carbamoyl Phosphate to Ornithine forming Citrulline
- Takes place in the mitochondria
- Subsequent steps takes place in the cytoplasm.

Transporters of Urea Cycle

- **Ornithine Transporter:** For entry of Ornithine
- **Citrulline Transporter:** For exodus of Citrulline

Arginino Succinate Synthetase

- Links amino nitrogen of aspartate to citrulline
- Aspartate provides second nitrogen of Urea
- This enzyme is a Ligase
- This reaction requires **ATP**
- Two inorganic phosphates are utilized.

Arginino Succinate Lyase

Cleavage of Argino succinate to Arginine and Fumarate. This enzyme is a Lyase

Arginase[Q]

Hydrolytic cleavage of arginine, releases urea and reforms ornithine which reenter into mitochondria.

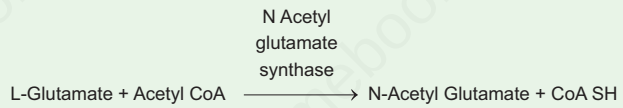
Arginase is a Hydrolase[Q]

Remember

All the enzymes in the Cytoplasm starts with the letter 'A'.

N-Acetyl Glutamate Synthase

- Enzyme which catalyses the formation of N Acetyl Glutamate (NAG).
- Generally considered as the sixth enzyme of Urea Cycle.
- Because **CPS-I is active only in the presence of NAG**[Q].



Energetics of Urea Cycle

- Urea cycle requires **4 high energy phosphates**
- Urea cycle requires 3 ATPS[Q] directly.

Urea Bicycle

Urea cycle is linked to TCA cycle through Fumarate and Aspartate. Hence this cycle is called **urea bicycle**.

Remember by Urea cycle

- CO_2, ATP, NH_4^+ and Aspartate consumed.
- Ornithine, Citrulline, Argininosuccinate, Arginine not consumed
- The compound that enter into urea cycle and regenerated is Ornithine

CLINICAL CORRELATIONS: UREA CYCLE DISORDERS

Key Points of Urea Cycle Disorders

Characterized by

- Hyperammonemia
- Encephalopathy
- Respiratory alkalosis.

Clinical Symptoms common to all Urea Cycle Disorders

In the neonatal period

Symptoms and signs are mostly related to brain dysfunction and are similar regardless of the cause of the hyperammonemia.

The affected infant is normal at birth but becomes symptomatic following the introduction of dietary protein.

- Refusal to eat
- Vomiting
- Tachypnea
- Lethargy
- Convulsions are common
- Can quickly progress to a deep coma.

In infants and older children

- Vomiting
- Neurologic abnormalities (ataxia, mental confusion, agitation, irritability, and combativeness)

Ammonia intoxication is most severe in the deficiency of first two enzymes.

Because once citrulline synthesized some ammonia is already been covalently linked to an organic metabolite.

BIOCHEMICAL DEFECT IN UREA CYCLE DISORDERS

Urea cycle disorders due to enzyme deficiency	
Disorder	**Enzyme defective**
Hyperammonemia Type-I	Carbamoyl Phosphate Synthetase I (CPS-I)
Hyperammonemia type-II	Ornithine Transcarba-moylase (OTC)
Citrullinemia Type I (Classic Citrullinemia)	Argino succinate synthetase
Arginosuccinic aciduria	Arginosuccinate lyase
Hyperargininemia	Arginase
Urea cycle disorders due to transporter defect	
Citrullinemia Type II	Citrin (transport aspartate and glutamate) defect
Hyperammonemia-hyperornithinemia-homocitrullinuria (HHH) syndrome	Ornithine transporter defect

HIGH YIELDING FACTS—UREA CYCLE DISORDERS

Hyperammonemia Type II (OTC Deficiency)

- Most common Urea Cycle disorder[Q]
- Disorder with **X-linked partially dominant inheritance** (All other Urea Cycle Disorders are Autosomal Recessive)
- Urea cycle disorder with Orotic Aciduria
- Marked elevations of plasma concentrations of glutamine and alanine with low levels of citrulline and arginine
- Orotate may precipitate in urine as a pink colored gravel or stones.

Orotic aciduria in Hyperammonemia Type II

- Ornithine Transcarbamoylase defective hence Carbamoyl Phosphate accumulate in the mitochondria
- Carbamoyl Phosphate reaches the cytoplasm enter into Pyrimidine Synthesis
- Orotic Acid, an intermediate in the Pyrimidine synthesis accumulates which leads to Orotic Aciduria.

Arginosuccinic Aciduria

- Trichorrhexis nodosa (dry and brittle hair) is a common finding

Hyperargininemia (Argininemia), A Distinct Urea Cycle Disorder

- Hyperargininemia is the urea cycle disorder with least Hyperammonemia. Because by the time Arginine is formed the two nitrogen are already incorporated
- There are 2 genetically distinct arginases in humans
- One is cytosolic (ARG1) and is expressed in the liver and erythrocytes, and the other (ARG2) is found in renal and brain mitochondria
- The gene for ARG1, the enzyme that is deficient in patients with arginase deficiency
- The clinical manifestations of this condition are quite different from those of other urea cycle enzyme defects
- **A progressive spastic diplegia** with scissoring of the lower extremities, choreoathetotic movements, and loss of developmental milestones in a previously normal infant.

The compounds excreted in urine in hyperargininemia:

- Cystine, Ornithine, Lysine, Arginine [COLA]
- Alpha ketoguanidinovaleric acid.

N-Acetyl Glutamate Synthase Deficiency

- The sixth enzyme deficiency which lead to a urea cycle disorder
- The condition is almost similar to Hyperammonemia Type I

Contd...

Contd...

- Arginine[Q] an allosteric activator of NAG Synthase improves CPS-I defect as N-Acetyl Glutamate activates CPS-I
- But Arginine does not improve N-Acetyl Glutamate deficiency, as the enzyme itself is defective.

Hyperammonemia-Hyperornithinemia-Homocitrullinemia (HHH) Syndrome

- **Autosomal recessively inherited disorder**
- **Biochemical Defect is** mutation in the **ORNT 1 gene** that encodes mitochondrial membrane **Ornithine Permease**
- This results in defect in the transport system of ornithine from the cytosol into the mitochondria
- This leads to accumulation of ornithine in the cytosol causes **hyperornithinemia**
- Deficiency of ornithine in the mitochondria results in disruption of the urea cycle and **hyperammonemia**
- **Homocitrulline** is presumably formed from the reaction of mitochondrial carbamoyl phosphate with lysine.

Citrullinemia Type II

- The adult form (type II) is caused by the deficiency of a mitochondrial transport protein named citrin
- Citrin (aspartate-glutamate carrier protein) is a mitochondrial transporter encoded by a gene (SLC25A13) located on chromosome 7q
- One this protein's functions is to transport aspartate from mitochondria into cytoplasm
- Aspartate is required for converting citrulline to argininosuccinic acid
- So Citrulline accumulates.

Biochemical Investigation in a Case with Hyperammonemia

Normal Blood Ammonia level **20–40 µg/dl**

Methods of estimation of blood ammonia:

- Chemical Method: Berthelot Method
- Enzymatic method: Glutamate Dehydrogenase Method
- Using Ammonia selective Electrodes Methods of estimation of Urea

Methods of estimation of Urea:

- Chemical Method: Diacetyl Monoxime Thiosemicarbazide Method
- Enzymatic Method: Using Urease

Tandem Mass Spectrometry is the most sensitive tool to detect metabolic Disorders.

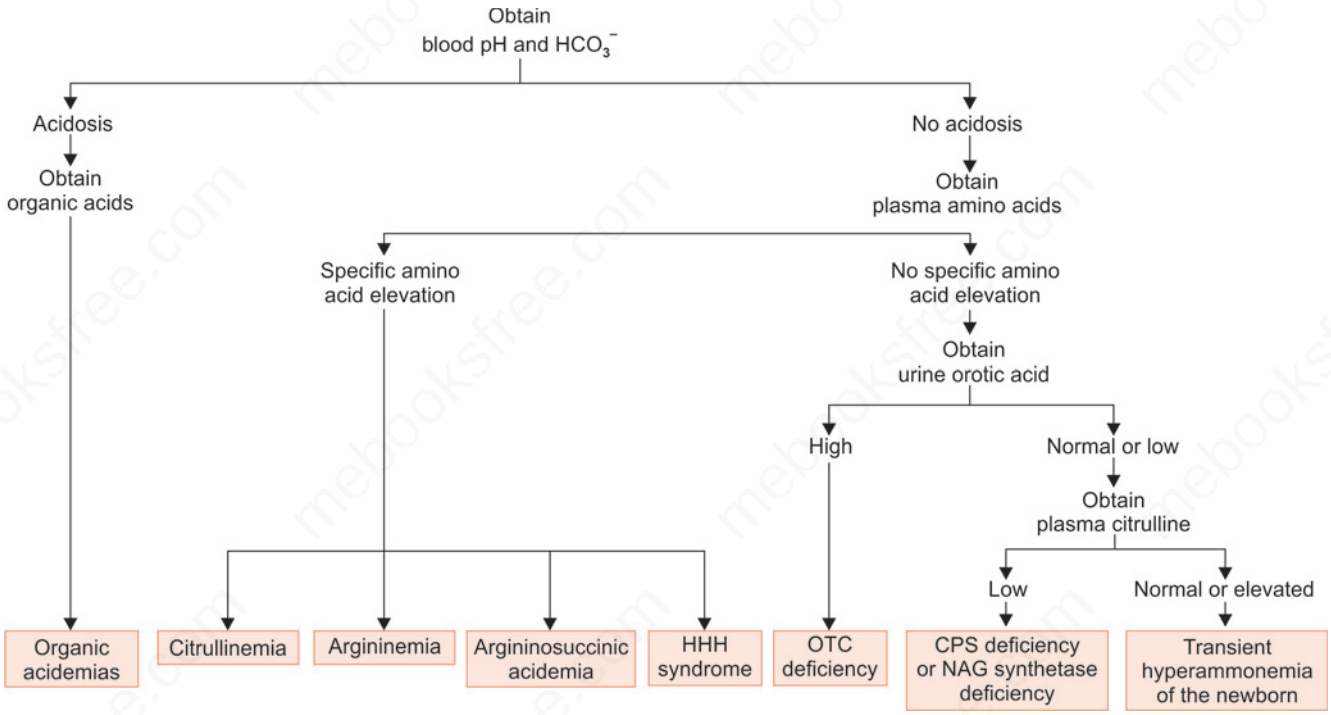


Fig. 1.13: Biochemical investigation of hyperammonemia

Biochemical Basis of Treatment of Urea Cycle Disorder

- **Arginine:**
  - Essential Amino Acid
  - Provide Ornithine
  - Arginine is an activator of N Acetyl Glutamate Synthase but contraindicated in Arginase Defect
- **Acylation therapy:** The main organic acids used for this purpose are sodium salts of benzoic acid and phenylacetic acid.

Principle: Exogenously administered organic acids form acyl adducts with endogenous nonessential amino acids.

These adducts are nontoxic compounds with high renal clearances.

- **Sodium Benzoate:** Benzoate forms hippuric acid with endogenous glycine in the liver. Each mole of benzoate removes 1 mole of ammonia as glycine.

Benzoic Acid + CoA → Benzoyl CoA + Glycine → Benzoyl Glycine [Hippuric Acid]

- **Sodium Phenylacetate:** Phenylacetate conjugates with glutamine to form phenylacetylglutamine, which is readily excreted in the urine. One mole of phenylacetate removes 2 moles of ammonia as glutamine from the body

Phenyl Acetic Acid + CoA → Phenyl Acetyl CoA + Glutamine → Phenyl Acetyl Glutamine.

INDIVIDUAL AMINO ACIDS

Phenylalanine and Tyrosine

Phenylalanine

- Aromatic amino acid
- Essential amino acid
- Hydrophobic amino acid
- Partly glucogenic partly ketogenic.

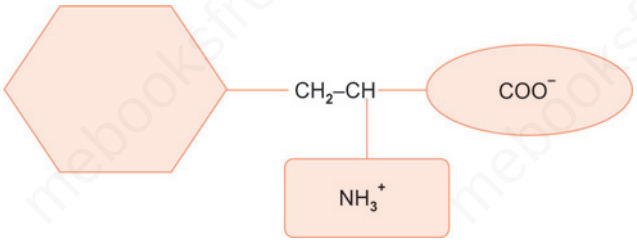


Fig. 1.14: Phenylalanine

Tyrosine

- Aromatic amino acid
- Synthesized from phenylalanine
- Nonessential
- *Partly glucogenic and partly ketogenic.*

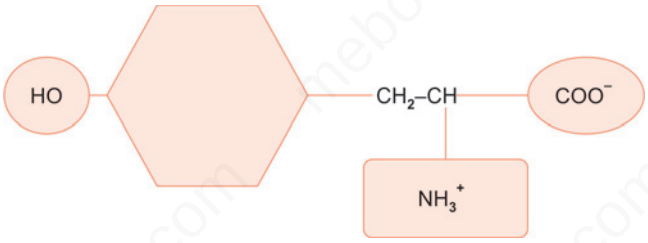


Fig. 1.15: Tyrosine

Synthesis of Tyrosine from Phenylalanine

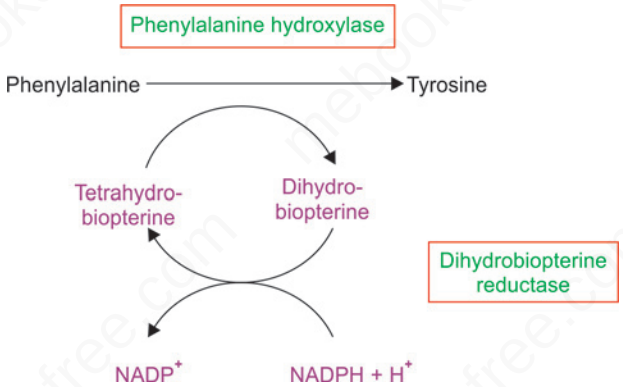


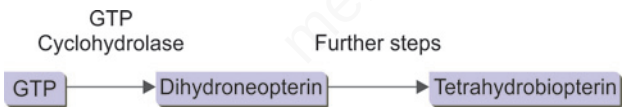
Fig. 1.16: Conversion of phenylalanine to tyrosine

Phenylalanine Hydroxylase

- Enzyme belongs to Mixed Function Oxidase (Monooxygenase)
- Require coenzymes Tetrahydrobiopterin, NADPH
- One mol of oxygen is incorporated
- Irreversible reaction.

Tetrahydrobiopterin

- Resemble folic acid but is not a vitamin
- Precursor of Tetrahydrobiopterin is Guanosine Triphosphate (GTP)
- Rate limiting enzyme in the pathway is GTP Cyclohydrolase.



Enzymes with Tetrahydrobiopterin as Coenzyme

- Phenylalanine Hydroxylase
- Tyrosine Hydroxylase
- Tryptophan Hydroxylase
- Nitric Oxide Synthase

CATABOLISM OF TYROSINE

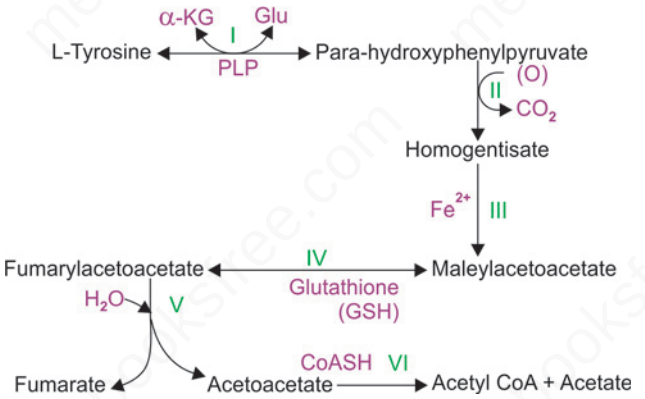


Fig. 1.17: Catabolism of tyrosine

Enzymes

i. Tyrosine Transaminase
ii. pHydroxyphenylpyruvate Hydroxylase
iii. Homogentisate Oxidase
iv. Maleylacetoacetate Cis-trans Isomerase
v. Fumaryl Acetoacetate Hydrolase

Important Points in the Catabolism of Tyrosine

As phenylalanine is converted to tyrosine, the degradative pathway is the same for both Phenylalanine and Tyrosine.

Tyrosine Transaminase

PLP is the coenzyme for this reaction

Concept

Catabolic pathway of almost all amino acids starts with a transamination (except those amino acid which do not undergo transamination). This is because amino group prevents oxidative breakdown of amino acids.

Para Hydroxyphenylpyruvate Hydroxylase (4 Hydroxy-Phenylpyruvate Dioxygenase)

- This enzyme belongs to Dioxygenase, i.e. incorporates both the atoms of oxygen
- Cofactor for this enzyme is Copper
- Ascorbic Acid is also needed for this reaction.

Homogentisate Oxidase

- Belongs to Dioxygenase
- Contains Iron at the active site.

Maleyl acetoacetate cis-trans isomerase

- Belongs to Isomerase
- Need Glutathione (GSH) as cofactor.

Specialized Products from Tyrosine (Very important Topic)

- Melanin
- Catecholamines
- Thyroxine

SYNTHESIS OF MELANIN

- Takes place in the **melanosome of melanocyte present in the deeper layers of epidermis.**
- Under the influence of MSH.
- Melanin gives pigmentation to the skin and hair.

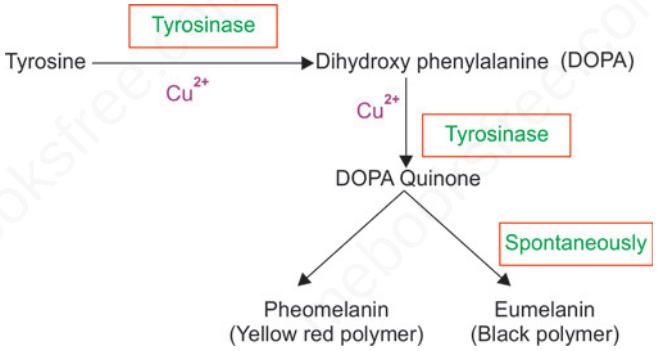


Fig. 1.18: Melanin synthesis

Tyrosinase

- **Rate limiting step**
- Monooxygenase
- Copper is the cofactor for this enzyme
- Single enzyme catalyse two reactions.

SYNTHESIS OF CATECHOLAMINES

Catecholamines are:

- Dopamine
- Epinephrine
- Norepinephrine

Catecholamines are compound which contain Catechol nucleus.

Site of Synthesis: Chromaffin cells of Adrenal Medulla and Sympathetic Ganglia.

- In adrenal medulla major product is Epinephrine (80%)
- In organs innervated by Sympathetic nerves major product is Norepinephrine (80%).

Remember
Epinephrine is also called Adrenaline, so adrenaline in adrenal medulla

Conversion of Tyrosine to Epinephrine involves 4 sequential steps:

1. Ring Hydroxylation
2. Decarboxylation
3. Side chain hydroxylation
4. N-Methylation

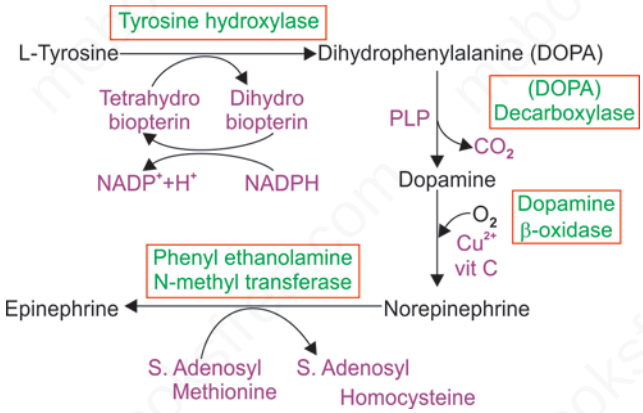


Fig. 1.19: Catecholamine

Important Points Catecholamine Synthesis

Tyrosine Hydroxylase

- Rate limiting step in catecholamine synthesis
- Similar to Phenylalanine hydroxylase
- Monooxygenase
- Require tetrahydrobiopterin.

Tyrosinase vs Tyrosine Hydroxylase

- Both the enzymes convert Tyrosine to DOPA
- Tyrosinase is expressed only in melanocyte where DOPA is used to synthesize Melanin
- Tyrosine Hydroxylase is expressed only in the sites where catecholamines are synthesized, where DOPA utilized for Catecholamine synthesis
- Tyrosinase is a monooxygenase containing Cu^{2+} in the active site
- Tyrosine Hydroxylase is a monooxygenase with Tetrahydrobiopterin as the cofactor.

DOPA Decarboxylase

- Present in all the tissues
- PLP is the coenzyme for this enzyme[Q].

DEGRADATION OF CATECHOLAMINES

- The half life of catecholamines are very short, only 2–5 minutes

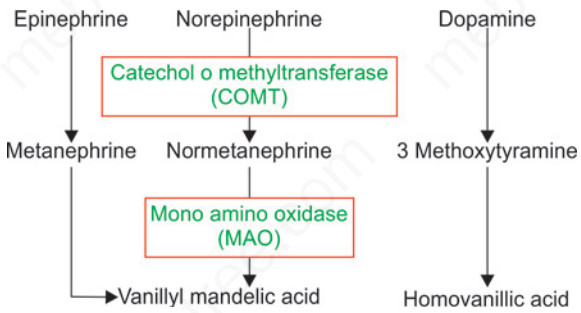


Fig. 1.20: Degradation of catecholamines

- Epinephrine and norepinephrine is catabolized by **Catechol O Methyl Transferase (COMT)Q then by Monoamino Oxidase (MAO)Q**
- The major end product of epinephrine and norepinephrine is **Vanillyl Mandelic Acid (VMA)Q**
- Normal level of VMA excretion in urine is 2–6 mg/24 hour
- **The major end product of Dopamine is** Homo Vanillic Acid (HVA).

Synthesis of Thyroid Hormones

Thyroid hormones are synthesized on thyroglobulin, a large iodinated glycosylated protein. It contains. **115 tyrosine residues.**

Tyrosine residues are iodinated to form Mono-Iodo–Tyrosine (MIT) and Di-iodo Tyrosine (DIT). Coupling of **MIT and DIT** on the thyroglobulin produce Thyroxine

- MIT + DIT → Tri-iodothyronine (T3)
- DIT+DIT → Tetra-iodothyronine. (T4) or Thyroxine.

CLINICAL CORRELATIONS (PHENYLALANINE AND TYROSINE METABOLISM)

Metabolic Disorders associated with catabolic pathway of Phenyl alanine and Tyrosine
- Phenylketonuria
- Alkaptonuria
- Tyrosinemias
- Hawkinsinuria
- Segawa syndrome

Disorders associated with melanin synthesis
- Albinism

Disorders associated with excess Catecholamines
Pheochromocytoma

Metabolic Disorders Associated with Catabolic Pathway of Phenylalanine and Tyrosine

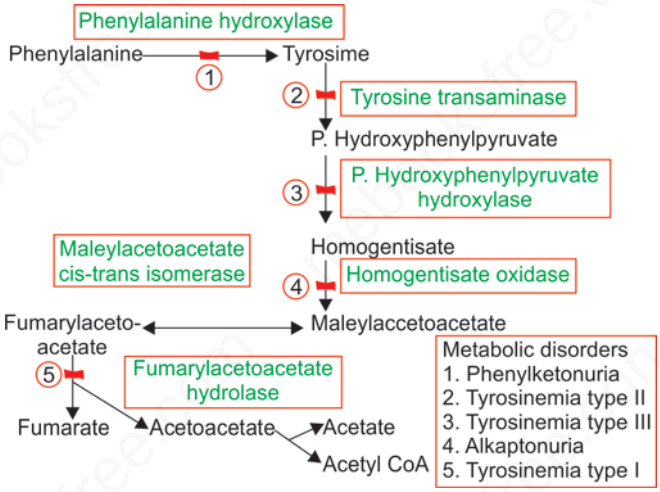


Fig. 1.21: Biochemical defects in the metabolism of aromatic amino acids

PHENYLKETONURIA

Classic Phenylketonuria (Type I PKU)

- Most common metabolic disorder concerned with amino acid

Biochemical Defect

- Phenylalanine Hydroxylase Deficiency.
- Phenylalanine could not be converted in to Tyrosine.
- Phenylalanine in the blood rises.
- Alternate metabolic pathways are opened.

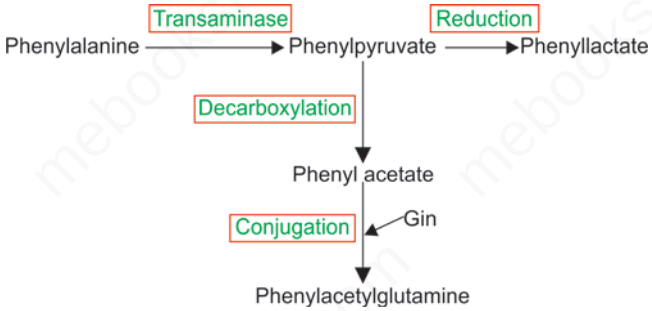


Fig. 1.22: Alternate metabolic pathways in PKU

The reason for the name Phenylketonuria
Excess phenylalanine is metabolized to phenylketones (phenylpyruvate and phenylacetate) that are excreted in the urine, giving rise to the term phenylketonuria **(PKU).**

Clinical Presentation of Phenylketonuria

- The affected infant is normal at birth
- Profound intellectual disability develops gradually if the infant remains untreated
- Vomiting, sometimes severe enough to be misdiagnosed as pyloric stenosis
- Older untreated children become hyperactive with autistic behaviors, including purposeless hand movements, rhythmic rocking, and athetosis
- The infants are lighter in their complexion than unaffected siblings. (Phenylalanine not converted to Tyrosine, so decreased melanin synthesis)
- These children have an unpleasant mousey or musty odor of **phenylacetic acid.**Q JIPMER 2015

The brain is the main organ affected by hyperphenylalaninemia.
The CNS damage in affected patients is caused by the elevated concentration of phenylalanine in brain tissue. The high blood levels of phenylalanine in PKU saturate the transport system across the blood-brain barrier causing inhibition of the cerebral uptake of other large neutral amino acids such as tyrosine and tryptophan.

*Lab Diagnosis of PKU*Q

Guthries Test (Bacterial Inhibition Assay of Guthrie)

- Rapid screening Test in the blood sample
- First method used for this purpose

- Certain strains of Bacillus Subtilis need Phenylalanine as an essential growth factor
- Bacterial growth is proportional to blood phenylalanine.

Ferric Chloride Test

- **Screening** test in urine sample
- **Identifies Phenylketones in urine sample**
- A simple test for diagnosis of infants with developmental and neurologic abnormalities
- Nowadays it has no place in any screening program especially in developed countries
- These tests have been replaced by more precise and quantitative methods (**fluorometric and tandem mass spectrometry).**

Tandem Mass Spectrometry

- The method of choice is tandem mass spectrometry, which identifies all forms of hyperphenylalaninemia.

Other methods

- Molecular Biology Techniques like **Phenylalanine Hydroxylase specific probes**
- Quantitative measurement of Blood Phenylalanine. (Blood level > 20 mg/dl in PKU)
- Enzyme assay in dry blood spot also done.

Treatment of Classical PKU

- *A low–phenylalanine diet*
- Administration of large neutral amino acids (LNAAs) is another approach to diet therapy.
- **Sapropterin dihydrochloride (Kuvan)**, a synthetic form of BH4, which acts as a cofactor in patients with residual PAH activity, is approved by the FDA to reduce phenylalanine levels in PKU.
- Preliminary trials with recombinant phenylalanine ammonia lyase have been encouraging and demonstrated reduced blood levels of phenylalanine during treatment.

Rationale for using Large Neutral Amino Acids (LNAAs) as treatment for PKU

- LNAAs (tyrosine, tryptophan, arginine, leucine, isoleucine, valine, methionine, histidine, lysine, threonine and phenylalanine) share the same transporter protein (LNAA type 1, LAT-1 for transit through the intestinal cell membrane and blood-brain barrier.
- Binding of LNAA to the transporter protein is a competitive process.
- The rationale for use of LNAA is that these molecules compete with phenylalanine for transport across the blood-brain barrier; therefore, large concentrations of other LNAAs in the intestinal lumen and in the blood reduce the uptake of phenylalanine into bloodstream and the brain.

Nonclassical Phenylketonuria

Biochemical Defect

Hyperphenylalaninemia due to Tetrahydrobiopterin defect

- Due to Dihydrobiopterin Reductase^{Q} Defect (Type II and Type III PKU)
- Due to defect in the enzymes that synthesize Tetrahydrobiopterin **(Type IV & Type V PKU)**
  - 6-pyruvoyltetrahydropterin synthase, (Most Common)
  - Guanosine Triphosphate (GTP) Cyclohydrolase.

Lab Diagnosis of Nonclassical PKU

- Meausurement of Neopterin and Biopterin [Oxidative Product of Dihydrobiopterin and Tetrahydrobiopterin] level in urine
- Tetrahydrobiopterin (BH4)loading test normalizes Plasma Phenylalanine level
- Enzyme Assay in dry blood spots on filter paper
- Genetic test: Mutation analysis and deletion/duplication studies are clinically available for all these enzyme defects and help to confirm the diagnosis.

Segawa Syndrome (Hereditary Progressive Dystonia)

- Tetrahydrobiopterin deficiency due to defect in the enzyme GTP Cyclohydrolase
- But interestingly no Hyperphenylalaninemia
- Autosomal Dominant Inheritance
- Dystonia with diurnal variation
- Females are affected more than males.

Alkaptonuria

- Autosomal recessive disorder
- 1st inborn error detected.
- Belongs to Garrod's Tetrad (Alkaptonuria, Albinism, Pentosuria, Cystinuria).

Biochemical Defect

- Homogentisate oxidase deficiency
- Accumulation of Homogentisic Acid (Homogentisate). which polymerizes to form Alkaptone bodies.

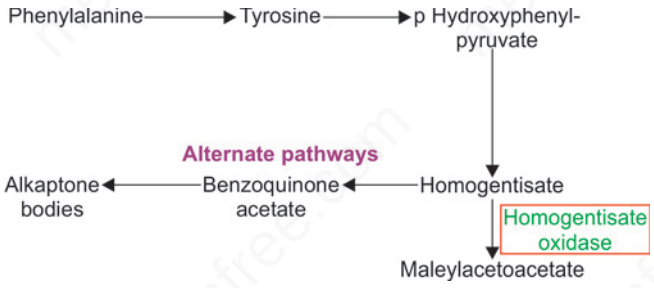


Fig. 1.23: Biochemical defect alkaptonuria

Clinical Presentation of Alkaptonuria

- Normal Life till 3rd or 4th decade
- Urine Darkens on standing is the only manifestation in children
- In adults **ochronosis,** i.e. Alkaptone bodies deposited in intervertebral disk, cartilage of nose, pinna, etc. leading to pigmentation.
- Arthritis
- NO MENTAL RETARDATION[Q].

Laboratory Diagnosis[Q]

- Alkalanization increase darkening of urine
- Benedicts test positive in urine because homogentisic acid is reducing agent
- Ferric chloride test positive
- Silver nitrate test positive.

Treatment

- New Drug is Nitisinone [NTBC] which inhibit para Hydroxylphenylpyruvate hydroxylase which leads to the accumulation of homogentisic acid
- Symptomatic treatment.

Tyrosinemia

There are three types of Tyrosinemias Type I, Type II and Type III

Type I (Hepatorenal Tyrosinemia, Hereditary Tyrosinemia)

Biochemical defect:

- Fumarylacetoacetate hydrolase deficiency
- Organs affected are liver, kidney, and peripheral nerves
- Organ damage is believed to result from accumulation of metabolites of tyrosine degradation, especially fumarylacetoacetate and succinylacetone
- Cabbage like odor due to Succinylacetone.

Diagnosis

- Plasma tyrosine value less diagnostic value. Because it is dependent on diet
- Elevated Succinylacetone in urine and blood is more diagnostic.

Treatment

- Diet low in Phenylalanine and Tyrosine
- New Drug is Nitisinone [NTBC].

Type II Tyrosinemia (Oculocutaneous Tyrosinemia, Richner-Hanhart Syndrome)

Biochemical defect

- Tyrosine transaminase deficiency.

Clinical manifestations

- Palmar and plantar hyperkeratosis
- Herpetiform corneal ulcers
- Intellectual disability.

Type III (Neonatal Tyrosinemia)

Biochemical defect

Parahydroxylphenylpyruvate Hydroxylase (4 Para Hydroxyphenylpyruvate Dioxygenase 4-HPPD deficiency.

Remember
First tyrosinemia defective enzyme's name also start with letter **F**, fumarylacetoacetate hydrolase deficiency.
Type **t**wo tyrosinemia defective enzyme also start with **T and T,** i.e. tyrosine transaminase.

Hawkinsinuria

- Hawkinsinuria is inherited as an autosomal dominant trait
- Certain missense mutations in the gene for Para hydroxylphenylpyruvate hydroxylase) (4 Para Hydroxyphenylpyruvate Dioxygenase)
- This results in an abnormal enzyme activity
- The mutant enzyme, incapable of normally oxidizing 4-hydroxyphenylpyruvate to homogentisic acid
- Instead it forms an intermediate that reacts with cysteine to form the unusual organic acid hawkinsin
- Hawkinsin named after the first affected family secondary glutathione deficiency may occur
- An unusual odor (described as like that of a swimming pool).

Disorders Associated With Excess Catecholamines

- Pheochromocytoma
- Paraganglioma
- Pheochromocytoma-Associated Syndromes
- Neurofibromatosis type 1 (NF 1)
- Multiple endocrine neoplasia type 2A and type 2B (MEN 2A, MEN 2B)

Pheochromocytoma

Symptomatic catecholamine-producing tumors, in **adrenal and extraadrenal** retroperitoneal, pelvic, and thoracic sites.

Clinical Presentation

The classic triad of Pheochromocytoma:

- Episodes of palpitations
- Headaches
- Profuse sweating are typical and constitute a classic triad
- All three symptoms are associated with hypertension.

Biochemical Testing of Pheochromocytoma and paraganglioma

Elevated plasma and urinary levels of:

- Catecholamines
- Metanephrines
- Vanillyl Mandelic acid

Biochemical methods used for pheochromocytoma and paraganglioma diagnosis		
Diagnostic method	**Sensitivity**	**Specificity**
24 hour Urinary Testing		
1. Vanillylmandelic acid	++	++++
2. Catecholamines	+++	+++
3. Fractionated metanephrines[a]	++++	++
4. Total metanephrines	+++	++++
Plasma Tests		
1. Catecholamines	+++	++
2. Free metanephrines	++++	+++

Albinism

Disorder associated with deficiency of Tyrosinase enzyme which synthesize Melanin, the pigment of skin and eye.

Classification of Albinism

Generalized Albinism or Oculocutaneous Albinism (OCA)

- **OCA-1** Tyrosinase deficient
- **OCA-2** Tyrosinase positive (Most common albinism)
- **OCA-3** (Rufous, red OCA)

Syndromes associated with Oculocutaneous Syndrome

- Prader willi and Angelman syndrome
- Hermansky-Pudlak syndrome
- Chédiak-Higashi syndrome

Ocular Albinism

- Ocular albinism (Nettleship falls type)

Localized Albinism:

Seen in:

- Piebaldism
- Waardenberg Syndrome

TRYPTOPHAN

- Aromatic amino acid
- Essential amino acid
- Special group present is indole group
- Glucogenic and ketogenic.

Catabolic Pathway Tryptophan (Kynurenine -Anthranilate Pathway)

- Major metabolic fate of Tryptophan is to be oxidized by tryptophan pyrrolase

Tryptophan Pyrrolase (Tryptophan Oxygenase)

- Dioxygenase
- Iron Porphyrin Metalloprotein (i.e. it is a heme containing Protein).

Kynureninase

- Coenzyme is PLP.

Clinical Correlation-Kynurenine Anthranilate Pathway

Pellagra like symptoms in PLP deficiency

- Decreased Kynureninase leads to decreased NAD+ pathway
- Hence Niacin deficiency, which lead to Pellagra like symptoms.

Xanthurenate is excreted in urine in PLP deficiency

- This is because PLP deficieny leads to decreased Kynureninase activity
- Hence Kynurenine accumulate which is converted to Xanthurenate.

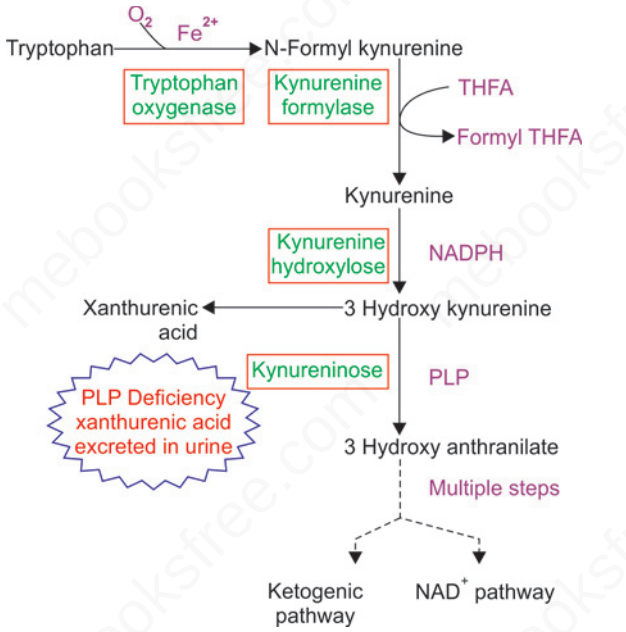


Fig. 1.24: Metabolic pathways of tryptophan

Specialized Products from Tryptophan

- Niacin (Nicotinic Acid)
- Serotonin
- Melatonin

Nicotinic Acid Pathway of Tryptophan

- 3% of Trptophan enter this pathway
- **60 mg Tryptophan is converted to 1 mg of Niacin**[Q]
- **Quinolinate Phosphoribosyl Transferase** is the rate limiting step in this pathway.

Serotonin (5 Hydroxytryptamine)

Synthesized in the Argentaffin cells in the intestine, mast cells, platelets and in the brain.

Functions of Serotonin

- Neurotransmitter in the brain
- Mood elevation
- GI motility
- Temp regulation
- Potent vasoconstrictor.

Melatonin

Synthesized in the Pineal gland.

Functions of Melatonin

- Diurnal variation
- Biological rhythm
- Sleep wake cycle

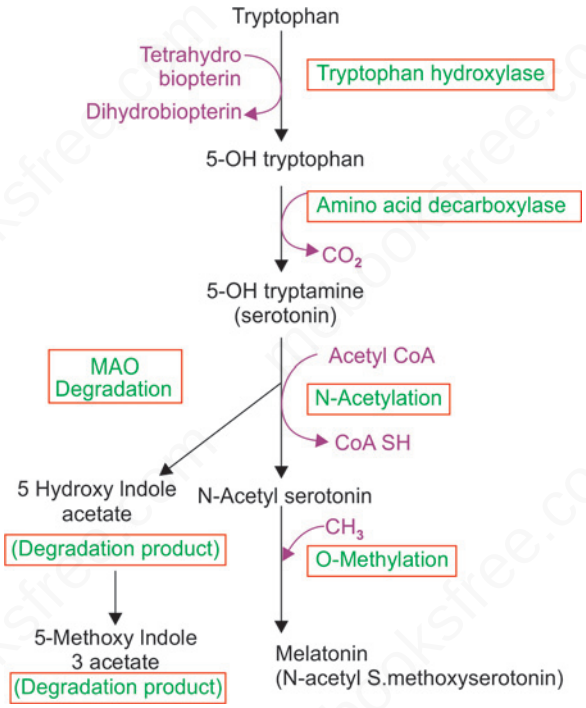


Fig. 1.25: Specialized products from tryptophan

Important Enzymes in the synthesis of Serotonin

Tryptophan Hydroxylase

- Rate limiting step in the serotonin and melatonin synthesis
- By this enzyme tryptophan is converted to 5 hydroxytryptophan
- Tetrahydrobiopterin is the coenzyme for this enzyme monoxygenase.

Hydroxylases dependent on Tetrahydrobiopterin

- Phenylalanine hydroxylase
- Tyrosine hydroxylase
- Tryptophan hydroxylase

Remember all are aromatic amino acids

Amino Acid Decarboxylase

- 5 OH Trytophan is decarboxylated to 5 OH Tryptamine, or Serotonin.

CATABOLISM OF SEROTONIN

- **Mono amino oxidase** is the enzyme
- **5 Hydroxy indole acetic acid (5HIAA)** is the degradatory product of Serotonin
- Normal urinary excretion of 5 HIAA is < 5 mg/day.

SYNTHESIS OF MELATONIN

- N Acetylation of serotonin followed by N-methylation in the pineal body forms Melatonin
- Methyl donor is S-Adenosyl Methionine (SAM)

Excretory Product of Tryptophan

Normal excretory product of Tryptophan in Urine is **5 Hydroxy Indole Acetate and Indole 3 Acetate**.

METABOLIC DISORDERS ASSOCIATED WITH TRYPTOPHAN METABOLISM

- Carcinoid Syndrome
- Hartnup's Disease
- Blue Diaper Syndrome

Carcinoid Tumor [Argentaffinoma]

- Belongs to gastrointestinal neuroendocrine tumors
- Tumor of Argentaffin Cells that secrete Serotonin.
- Increased Synthesis of Serotonin

Clinical Symptoms

- Most common symtoms are Intermittent Diarhea (32–84%) and Flushing (63–75%)
- Sweating
- Fluctuating hypertension
- Pellagra like symptoms

Diagnosis

- Serum serotonin increased
- Urinary 5 HIAA increased
- **Neuroendocrine markers used for diagnosis are:**
  - Chromogranin A
  - Neuron specific enolase
  - Synaptophysin

Typical and Atypical Carcinoid

Typical Carcinoid

- Is caused by a **midgut** carcinoid tumor
- Increased synthesis of 5 Serotonin
- Expanded serotonin pool size, increased blood and platelet serotonin
- Increased urinary 5-hydroxyindolacetic acid (5-HIAA).

Atypical Carcinoid

- **Foregut** carcinoids are the most likely to cause an atypical carcinoid syndrome.

Biochemical Defect

- Due to a deficiency in the enzyme aromatic amino acid decarboxylase
- 5-Hydroxy Tryptophan (5 HTP) cannot be converted to 5-Hydroxytryptamine (5HT) (serotonin)
- 5-Hydroxytryptophan is secreted into the blood-stream
- Plasma serotonin levels are normal
- Characteristically, urinary 5-Hydroxytryptophan and 5-Hydroxytryptamine (5-HTP is converted to 5- HT in the kidney) are increased
- But urinary 5-HIAA levels are only slightly elevated.

Tryptophan hydroxylase | Aromatic amino acid decarboxylase

Tryptophan ⟶ 5 Hydroxy tryptophan ⟶ 5 Hydroxy tryptamine

Hartnup Disorder

- Autosomal Recessive Condition
- Named after first family in which the disorder identified.

Biochemical Defect

- Defective absorption of **tryptophan and other neutral amino acid from intestine and renal tubules**
- The transporter protein for these amino acids (B0AT1) is encoded by the *SLC6A19* gene
- Two chemically close transcription factors, angiotensin-converting enzyme (ACE2) in the intestine and renal tubules, and collectrin in the renal tubules, are required for expression of B0AT1 transporter protein by the *SLC6A19* gene
- The mutated gene in patients with Hartnup disorder, unable to interact with the above transcription factors, results in deficiency of B0AT1 protein either in the intestine or in the renal tubules or in both.

Clinical Features

- Asymptomatic
- **Cutaneous Photosensitivity** is the most common presenting complaint
- Intermittent Ataxia manifested as unsteady wide based gait
- **Pellagra like symptoms.**

Pellagra Like Symptoms in Hartnup's Disorder

Decreased absorption of Tryptophan from intestine.

Decreased availability of Trp for NAD^+ pathway leading to Niacin deficiency.

Laboratory diagnosis of hartnup disease

- **Obermeyer Test** (Test for indole compounds in the urine) positive.

Treatment

- Lipid-soluble esters of amino acids and tryptophan ethyl ester
- Treatment with nicotinic acid or nicotinamide (50–300 mg/24 hr) and a high-protein diet.

Blue Diaper Syndrome (Drummond syndrome)

- Tryptophan is specifically malabsorbed
- The defect is expressed **only in the intestine** (unlike Hartnup Disease) and not in the kidney.

Blue Staining of the Diaper in Drummond Syndrome

This is due to bacterial breakdown of unabsorbed Tryptophan to Indican and IndigoBlue.

SIMPLE AMINO ACIDS

Glycine

- Simplest amino acid
- Nonessential
- Glucogenic
- Optically inactive amino acid.Q

Biosynthesis of GlycineQ

- Glycine Amino transferase catalyse the synthesis of Glycine from **Glyoxylate, Glutamate and Alanine**Q
- From **Serine** by Serine hydroxymethyltransferase. This is a reversible reaction

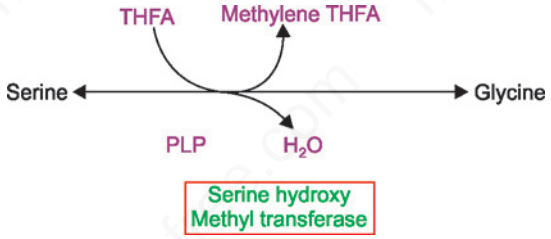


Fig. 1.26: Conversion of serine to glycine

- By **Glycine Synthase System** in Invertebrates
- From **Threonine** by Threonine Aldolase.

Serine Hydroxymethyltransferase
- Belongs to class II enzyme.
- Freely reversible
- Vitamins involved in conversion of Serine to Glycine are PLP and Folic Acid.[QDNB]
- When Serine is converted to Glycine, the β carbon [Q AIPGMEE] atom of Serine is donated to THFA.

Catabolism of Glycine

By Glycine Cleavage system[Q]

Present in liver mitochondria.

Glycine cleavage system consists of three enzymes and an H Protein that has covalently attached Dihyrolipoyl moiety. The three enzymes are:

- Glycine Dehydrogenase
- Aminomethyltransferase
- Dihydrolipomide Dehydrogenase
- The overall reaction is

Glycine + THFA + NAD+ $\rightarrow$ **CO_2 + NH3 + N5, N10 Methenyl THFA + NADH + H^+**

Specialized Products from Glycine
- Creatine, Creatine Phosphate and Creatinine
- Heme
- Purine Nucleotide
- Glutathione

Glycine as Conjugating Agent

- Conjugation of Bile acid (Glycocholic Acid, Glycochenodeoxy Cholic Acid)
- Conjugation of Benzoic Acid

Glycine + Benzoyl CoA → Benzoyl Glycine (Hippuric Acid)

Glycine as Neurotransmitter

- Both excitatory and inhibitory neurotransmitter.

Glycine is the recurring Amino acid present in the Collagen

- Every third amino acid in Collagen is Glycine.

CREATININE

- Synthesized from 3 Amino Acids **(Glycine, Arginine and Methionine [Q])**

Steps of Synthesis of Creatinine

Step I Glycine Arginine Amido Transferase
- First step in the kidney
- **Guanidino** group of Arginine is transferred to Glycine to form Guanidinoacetic Acid.

Step II Guanidinoacetate Methyltransferase
- Second step in the Liver
- Creatine is formed
- S Adenosyl Methionine is the methyl donor

Step III Creatine Kinase
- Third step in the muscle
- Creatine phosphate is formed.

Step IV
- Occur spontaneously
- Creatinine is formed.

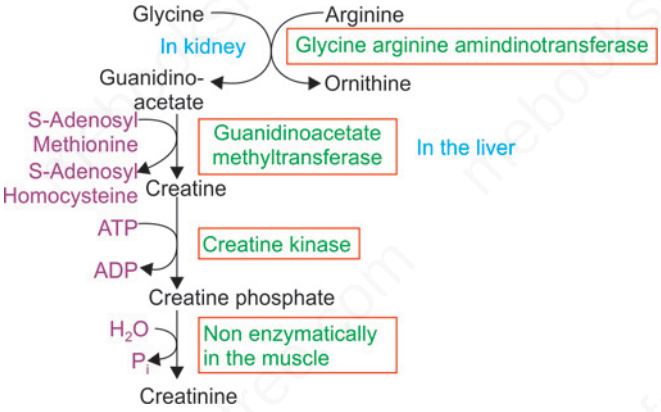


Fig. 1.27: Synthesis of creatinine

Heme

- Succinyl CoA + Glycine → Heme
- In the liver and erythroid precursor cells.

Formation of Purine Ring

- C4, C5, N7 of Purine ring is contributed by Glycine.

Remember glycine do not contribute to Pyrimidine Ring.[Q]

GLUTATHIONE[Q]

- Is otherwise called Gamma Glutamyl Cysteinyl Glycine
- Tripeptide[Q] from three Amino Acids: Glutamic Acid, Cysteine and Glycine
- Pseudopeptide[Q]
- Abbrevated as GSH
- Business part of Glutathione is Sulfhydryl group of Cysteine.

Functions of Glutathione[Q]

- **Meister's Cycle or Gamma Glutamyl Cycle**
  - Absorption of neutral amino acids in the intestine, kidney tubules and brain.
  - 3 mols of ATP utilized for the transport of amino acid.

- **Free Radical Scavenging**
  - Especially in the RBC, hence responsible for RBC membrane integrity.Q

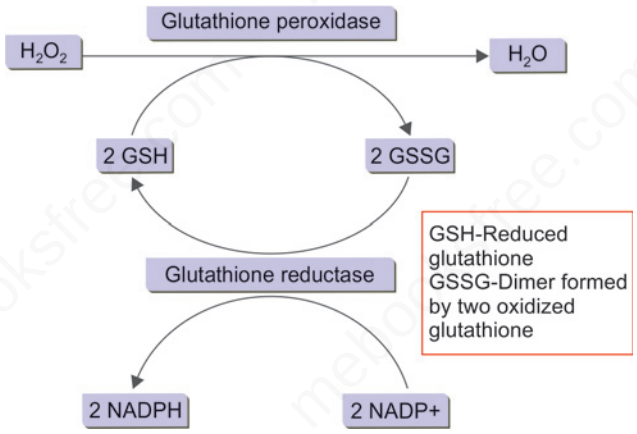


Fig. 1.28: Free radical scavenging

- **Reduction of Methemoglobin**Q
  - Keep the iron in the heme in the ferrous state by reduced glutathione.
- **Conjugation reactions in Phase II Xenobiotic reactions**
  - Glutathione S transferase is the enzyme.
- Acts as coenzyme for some reactions.

Remember
- Sarcosine is N-Methyl Glycine
- Betaine is Trimethyl Glycine

METABOLIC DISORDERS ASSOCIATED WITH GLYCINE
- Primary Hyperoxaluria Type I
- Primary Hyperoxaluria Type II
- Nonketotic Hyperglycinemia

Primary Hyperoxaluria Type I

- The most common form of primary hyperoxaluria.

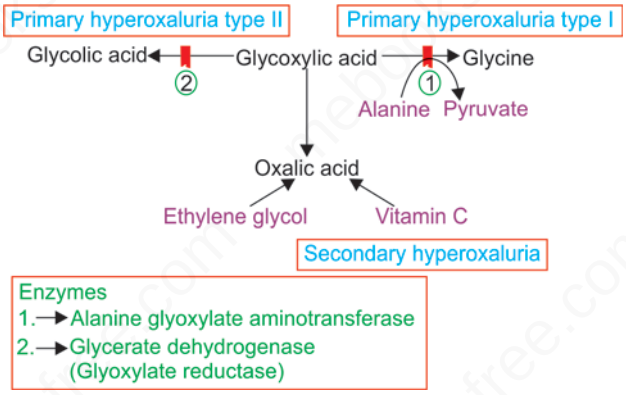


Fig. 1.29: Biochemical defect in hyperoxaluria

- It is due to a deficiency of the peroxisomal enzyme alanine-glyoxylate aminotransferase, (expressed only in the liver peroxisomes and requires pyridoxine (vitamin B6) as its cofactor).
- Protein targeting defect.

Primary Hyperoxaluria Type II (Glyceric Aciduria)

- Due to a deficiency of D-glycerate dehydrogenase (glyoxylate reductase enzyme complex).

Secondary Hyperoxaluria

- Pyridoxine deficiency (cofactor for alanine-glyoxylate aminotransferase)
- After ingestion of ethylene glycol
- High doses of vitamin C
- After administration of the anesthetic agent methoxyflurane (which oxidizes directly to oxalic acid)
- In patients with inflammatory bowel disease or extensive resection of the bowel (*enteric hyperoxaluria*).

Nonketotic Hyper Glycinemia

- Due to a defect in the Glycine Cleavage System.

ALANINE

- Simple amino acid
- Nonessential amino acid
- Principal glucogenic amino acid.
- Transports amino group from skeletal muscle.Q
- Participate in glucose-alanine cycle (Cahill cycle).

Biosynthesis of Alanine

- From Pyruvate by Transamination.

SERINE

- Hydroxyl group containing amino acid
- Glucogenic amino acid
- Nonessential amino acid
- Polar amino acid

Biosynthesis of Serine

- From glycine by serine hydroxymethyl transferase. PLP is a coenzyme in this reaction
- From glycolytic intermediate 3 Phosphoglycerate.

Remember
Vitamins required for conversion of serine to glycine are folic acidQ and pyridoxine.Q

Metabolic Functions of Serine

- Primary donor of one carbon group

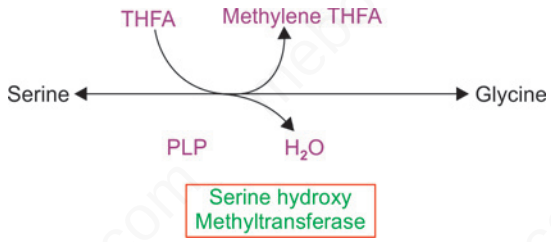


Fig. 1.30: Conversion of serine to glycine

- Serine is used for formation of cysteine
  - Serine + homocysteine cysteine + homoserine
- For phospholipid synthesis
  - Phosphatidyl serine
- Serine analogs as drugs
  - Cycloserine: Antituberculous drug
  - Azaserine: Anticancer drug
- Serine is used for:
  - Ethanolamine Synthesis
  - Choline (Trimethylethanolamine) synthesis
  - Betaine (Trimethylglycine) synthesis

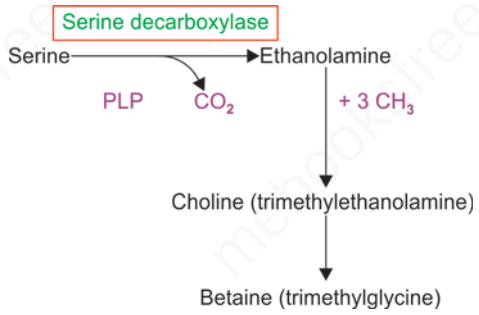


Fig. 1.31: Synthesis of betaine

- Serine is the precursor of Selenocysteine[QDNB/AIPGMEE]
- Serine used for Glycoprotein Synthesis-O Glycosylation takes place at Serine and Threonine residues
- Most common sites for Phosphorylation are Serine and Threonine
- Serine and Palmitoyl CoA are the starting material for the synthesis of Sphingosine, thereby Ceramide

Remember
All Sphingolipids are formed from Ceramide

SULFUR CONTAINING AMINO ACIDS

They are Methionine and Cysteine

Methionine

- Sulfur Containing Amino Acid
- Essential Amino Acid
- Glucogenic Amino Acid.

Cysteine

- Nonessential Amino Acid
- **Glucogenic Amino Acid.**

Specialized Products Derived from Cysteine[QDNB]
- Cysteine on decarboxylation gives Betamercaptoethanolamine.
- Coenzyme A
- Taurine
- Glutathione
- Cystine: Condensation product of two Cysteine.

Remember
- Serine on decarboxylation gives Ethanolamine
- Cysteine on decarboxylation gives Betamercaptoethanolamine

The Amino Acids that Decreases Aging

- Cysteine, hence aging is otherwise called Cysteine deficiency Syndrome
- Taurine.

The Amino Acid that Accelerate Ageing

- Homocysteine.

METABOLISM OF SULFUR CONTAINING AMINO ACIDS

Steps of Methionine metabolism

- Conversion of Methionine to S-Adenosyl Methionine (SAM) and Transmethylation reactions
- S Adenosyl Methionine to Homocysteine

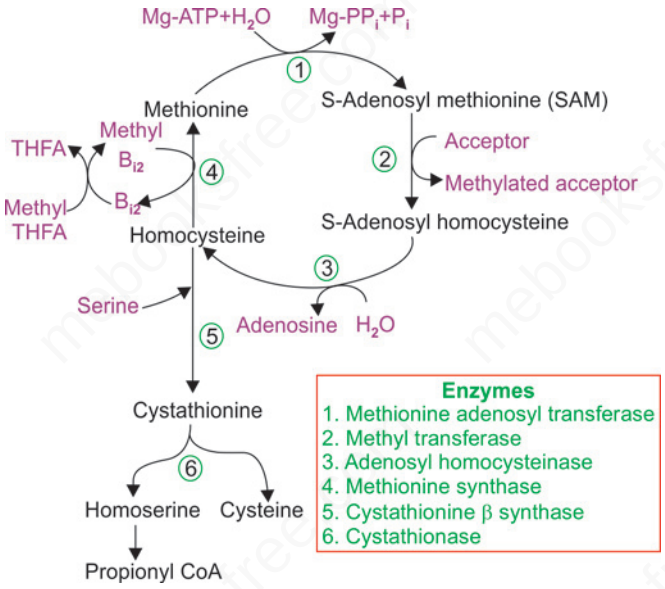


Fig. 1.32: Metabolism of sulfur containing amino acid

- Two fates of Homocysteine:
  a. Synthesis of Cysteine
  b. Resynthesis of Methionine
- Degradation of Cysteine.

Step I Methionine Adenosyl Transferase (MAT)

- Methionine converted to S-adenosyl Methionine, the principal methyl donor of the body
- ATP donates the Adenosyl group to methionine
- **3 Isoenzyme** [QDNB] forms for MAT are MAT-I, MAT-II and MAT-III
- MAT-I and MAT-III in the liver. MAT-II in the extrahepatic tissue.

Methionine has to be activated to S-Adenosyl Methionine (SAM)
In methionine, the thioether linkage (C-S-C) is very stable. Adenosyl group is transferred to Sulfur atom makes the methyl group labile. Hence methyl group can be easily transferred to acceptors.

Step II Fates of Homocysteine

1. **Resynthesis of Methionine**

- By transferring a methyl group to Homocysteine, Methionine is resynthesized
- **N5 Methyl THFA** and **Vitamin B_{12}** is involved. Folate trap is discussed below.

2. **Synthesis of Cysteine (Trans sulfuration reactions) Cystathionine Beta synthase**

- Homocysteine condenses with Serine to form Cystathionine by removing a H_2O by the enzyme Cystathionine Beta Synthase
- PLP is the coenzyme.

Cystathionase

- Cystathionine to Cysteine and Homoserine by Cystathionase
- PLP is the coenzyme
- By further reactions Homoserine is converted to Propionyl CoA then to Succinyl CoA.

Functions of S-Adenosyl Methionine

- Transmethylation reactions
- DNA Methylation
- Polyamine Synthesis.

Transmethylation Reactions

Acceptor of methyl group	Methylated compound
Guanidinoacetate	Creatine
Norepinephrine	Epinephrine
Epinephrine	Metanephrine

Contd...

Contd...

Acceptor of methyl group	Methylated compound
Ethanolamine	Choline
Carnosine	Anserine
Acetyl Serotonin	Melatonin

POLYAMINE SYNTHESIS

Polyamines are organic compounds having multiple amino groups. They are:

- **Cadaverine** derived from decarboxylation of Lysine
- **Putrescine** derived from decarboxylation of Ornithine
- **Spermidine** derived from Ornithine and Methionine
- **Spermine** derived from Ornithine and Methionine.

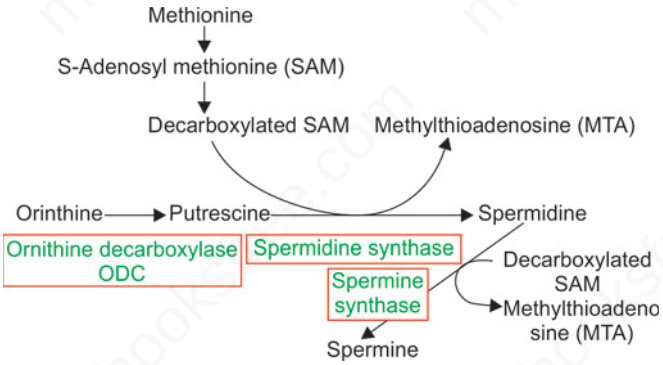


Fig. 1.33: Synthesis of polyamines

Steps of Polyamine Synthesis

- Ornithine is decarboxylated to form Putrescine, by the enzyme ornithine decarboxylase. Ornithine decarboxylase is the rate limiting step of Polyamine synthesis
- S adenosyl Methionine is decarboxylated to form Decarboxylated SAM
- Decarboxylated SAM donates 3 carbon atom and 1 α amino group to Putrescine to form Spermidine
- Decarboxylated SAM donates 3 carbon atom and 1 α amino group to Spermidine to form Spermine.

Significance of Polyamines

- They bear multiple positive charges, they associate readily with DNA and RNA
- Function in cell proliferation and growth
- Act as growth factors for cultured mammalian cells
- Stabilize intact cells and membranes and orgenelles
- Role in carcinogenesis.

Methionine can be synthesized from Homocysteine but it is an essential Amino Acid.
This is because Homocysteine is derived from Methionine

Vitamins in the metabolism of Sulfur containing Amino Acids

- Three vitamins needed are Vitamin B_{12}, Folic Acid and Vitamin B_6
- Vitamin B_{12} and Folic Acid for Methionine Synthase reaction.
- **Vitamin B_6 for Cystathionine Beta Synthase** (Transsulfuration reaction) and Cystathionase.

Folate Trap (THFA Starvation)

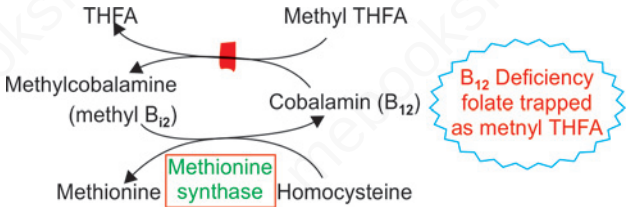


Fig. 1.34: Folate trap (THFA Starvation)

In Vitamin B_{12} deficiency the conversion of N5 Methyl THFA to THFA is blocked. This is the only reaction in which free THFA is released. Most of the body Folate is trapped as N5 Methyl THFA. This is called Folate Trap. This results in unavailability of free THFA for one carbon metabolism. Hence, this is also called **THFA Starvation.**

- **In Folate trap, the folic acid is trapped as its** Methyl derivative [QDNB]

In Vitamin B12 deficiency Homocysteine cannot be converted to Methionine. Hence Homocysteine accumulate. This is a risk factor for **acute coronary Syndrome.**

METABOLIC DISORDERS ASSOCIATED WITH SULFUR CONTAINING AMINO ACIDS

- Homocystinuria:
  - Classic Homocystinuria
  - Nonclassic Homocystinuria.
- Cystathioninuria (Cystathioninemia)
- Hypermethioninemia

Defective reabsorption:

- Cystinuria
- Oasthouse Syndrome

Lysosomal Storage Disorder:

- Cystinosis

Classic Homocystinuria

Most common inborn error of methionine metabolism.

Biochemical defect

- Due to deficiency of Cystathionine Beta Synthase.
- Homocysteine is not converted to Cysteine, so there is cysteine deficiency
- More homocysteine is available for methionine synthesis, so there is hypermethioninemia.

Clinical Features

- Normal at birth.
- Symptoms during infancy are nonspecific and may include failure to thrive and developmental delay.
- The diagnosis is usually made after 3 years of age, when subluxation of the ocular lens **(ectopia lentis)** occurs. This causes severe myopia and iridodonesis (quivering of the iris).
- Progressive **intellectual disability** is common.
- **Skeletal abnormalities** resembling those of **Marfan syndrome** tall and thin, with elongated limbs and arachnodactyly scoliosis, pectus excavatum or carinatum, genu valgum, pes cavus, high-arched palate, and crowding of the teeth are commonly seen.
- These children usually have fair complexions, blue eyes, and a peculiar malar flush.
- **Thromboembolic episodes** involving both large and small vessels, especially those of the brain, are common and may occur at any age.

Diagnosis

- Elevations of both methionine and homocystine (or homocysteine) in body fluids are the diagnostic
- Cystine level is low in the plasma
- Screening test for Homocystinuria-Cyanide Nitroprusside Test[Q] in freshly voided urine as homocystine is highly unstable
- Enzyme analysis in liver biopsy specimen or cultured fibroblasts
- DNA mutation analysis can be done
- Prenatal diagnosis by Enzyme assay of cultured amniotic cells or chorionic villi or by DNA analysis.

Treatment

- High doses of **vitamin B_6** (200–1,000 mg/24 hours) causes dramatic improvement in most patients
- Some patients do not respond to Vitamin B_6, may be due to Folate depletion. For them folic acid (1–5 mg/24 hours) has been added to the treatment regimen
- Restriction of methionine intake in conjunction with cysteine supplementation is recommended for patients who are unresponsive to vitamin B_6
- Betaine (trimethylglycine) lowers homocysteine levels in body fluids by remethylating homocysteine to methionine
- Administration of large doses of vitamin C (1 g/day) has improved endothelial function.

Nonclassic Homocystinuria

Can be due to:

- Defects in Methylcobalamin formation
- Deficiency of Methylene tetrahydrofolate Reductase (MTHFR).

Homocystinuria due to defect in Methylcobalamin formation:

- Methylcobalamin is the cofactor for the enzyme methionine synthase, which catalyzes remethylation of homocysteine to **methionine**
- Homocysteine cannot be remethylated to Methionine
- Homocysteine accumulate
- Methionine level decreases.

Laboratory findings

- **Megaloblastic anemia:** The presence of megaloblastic anemia differentiates Methylcobalamin formation defects from homocystinuria due to methylenetetrahydrofolate reductase deficiency
- Homocystinuria
- Hypomethioninemia.

Remember

The absence of hypermethioninemia differentiates nonclassical homocystinuria from cystathionine β-synthase Deficiency.

Treatment

Vitamin B_{12} in the form of hydroxycobalamin (1–2 mg/24 hours) is used to correct the clinical and biochemical findings.

Homocystinuria Caused by Deficiency of Methylenetetrahydrofolate Reductase

Due to Deficiency of Methylenetetrahydrofolate Reductase (MTHFR)

- This enzyme reduces N5, N10-methylenetetrahydrofolate to form 5-methyltetrahydrofolate
- N5 Methyl THFA provides the methyl group needed for remethylation of homocysteine to methionine
- Hence Hypomethionemia
- Homocystinemia and Homocystinuria
- Absence of megaloblastic anemia (Unlike Methyl-Cobalamin formation defect).

Treatment

- Combination of folic acid, vitamin B_6, vitamin B_{12}
- Methionine supplementation (Because Methionine is not resynthesized)
- Betaine (early treatment with betaine seems to have the most beneficial effect).

Cystathioninuria

Cystathionase Deficiency

Mental retardation, anemia, thrombocytopenia.

Cyanide nitroprusside test negative.

Cystinuria

- Included in Garrod's Tetrad (Cystinuria, Albinism, Pentosuria, Alkaptonuria)
- Defect in Dibasic Amino Acid Transporter
- Defective reabsorption of Cystine, Ornthine, Lysine and Arginine (Remember-COLA)
- **Cystine, Ornithine, Lysine and Arginine** (COLA)º in urine
- Cystine Stones in urine
- Cyanide Nitroprusside Test Positive
- Treated with ample hydration and alkalinization of urine.

Oasthouse Syndrome

- Malabsorption of Methionine and other Neutral Amino Acid.

Primary Hypermethioninemia

- Due to deficiency of hepatic Methionine Adenosyl Transferase (MAT I and III)
- MAT II present in other tissues are not defective
- Peculiar smell of Boiled Cabbage.

Cystinosis

- Lysosomal Storage Disorder
- Systemic disease caused by a defect in the metabolism of cystine
- Caused by mutations in the CTNS gene, which encodes a novel protein, cystinosin
- Cystinosin is a H^+ driven lysosomal cystine transporter
- Results in accumulation of cystine crystals in most of the major organs of the body:
  - Kidney
  - Liver
  - Eye
  - Brain.

Diagnosis

- Detection of cystine crystals in the cornea
- Confirmed by measurement of increased leukocyte cystine content.

Treatment

- Specific therapy is available with **cysteamine,** which binds to cystine and converts it to cysteine

- This facilitates lysosomal transport and decreases tissue cystine
- Kidney transplantation is a viable option in patients with renal failure.

BRANCHED CHAIN AMINO ACIDS

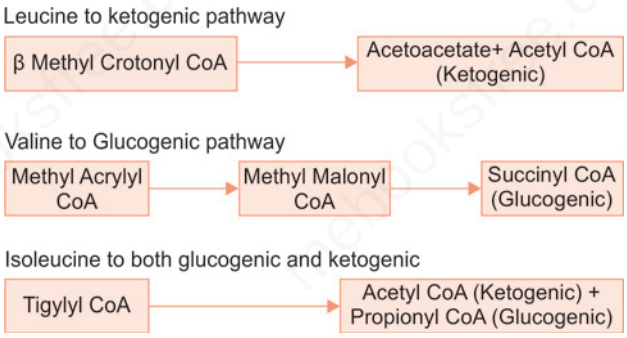
Branched chain amino acid	Metabolic fate
Valine	Glucogenic
Leucine	Ketogenic
Isoleucine	Both Ketogenic and Glucogenic

Remember
- Just like branches of a tree branched Amino Acid go into different metabolic fate
- All branched Amino Acids are essential.[α]

Three Common Steps in the Metabolism of Branched Chain Amino Acids

Reaction	Enzyme	Coenzyme
1. Transamination	Branched Chain Amino Acid Transaminase	PLP
2. Oxidative Decarboxylation	Branched Chain Keto Acid Dehydrogenase	Thiamine Pyrophosphate, FAD, NAD+, Lipomide and CoA
3. Dehydrogenation	Acyl CoA Dehydrogenase	FAD

After the First Three Common Steps



Main Metabolic Disorders Associated with Branched Chain Amino Acid
- Maple Syrup Urine Disease
- Isovaleric Aciduria

MAPLE SYRUP URINE DISEASE

Biochemical defect
- Deficiency of the enzyme Branched Chain Ketoacid Dehydrogenase
- Defective reaction is Defective Decarboxylation.

Components of Branched Chain Ketoacid dehydrogenase Complex and defective components in MSUD[Ω]

Gene	Component	MSUD types
E1α	Branched Chain α Ketoacid decarboxylase (contains TPP)	Type I A MSUD
E1β	Branched Chain α Ketoacid decarboxylase	Type I B MSUD
E2	Dihydrolipoyl Transacylase (contains Lipomide)	Type II MSUD
E3	Dihydrolipomide Dehydrogenase (Contains FAD)	Type III MSUD

Clinical features
- Affected infants who are normal at birth develop poor feeding and vomiting in the 1st week of life.
- Lethargy and coma, convulsions may ensue within a few days
- Metabolic Acidosis
- Physical examination reveals hypertonicity and muscular rigidity with severe opisthotonos
- Periods of hypertonicity may alternate with bouts of flaccidity manifested as repetitive movements of the extremities (boxing and bicycling)
- The peculiar odor of maple syrup (burnt sugar) found in urine, sweat, and cerumen
- Mental Retardation.

Lab diagnosis
- Plasma shows marked elevation of leucine, isoleucine, valine, and alloisoleucine (a stereoisomer of isoleucine not normally found in blood)
- Urine contains high levels of leucine, isoleucine, and valine and their respective ketoacids
- Ketoacids are detected **by Dinitrophenylhydrazine (DNPH)** Test
- Rothera's Test
- Enzyme analysis in leukocytes and cultured fibroblast
- Tandem Mass Spectrometry.

Treatment

Restrict Branched Chain Amino Acid
Give high doses Thiamine.

Isovaleric Aciduria

Biochemical defect

- Defective Leucine metabolism
- Defective Enzyme is Isovaleryl CoA Dehydrogenase
- Characteristic **odor of Sweaty Feet**ǫ is present.

Intermittent Branched Chain Ketonuria

- Retains some activity of Branched Chain α Ketoacid decarboxylase.

BASIC AMINO ACID

Lysine

- Represented by the letter K
- Essential Amino Acid
- Saccharopine is an intermediate in the Lysine Catabolic pathway
- Amino Acid deficient in Cereals
- Predominantly Ketogenic.

Functions of Lysine

- Hydroxy Lysine is important in Covalent Cross links in Collagen and Desmosine crosslinks in Elastin
- ε Amino group of Lysine forms Schiff's bases
- Lysine along with Methionine (SAM is the methyl donor) are the precursors of Carnitine
- Bacterial Putrefaction (Decarboxylation) of Lysine forms Cadaverine
- Histone Proteins are Lysine rich.

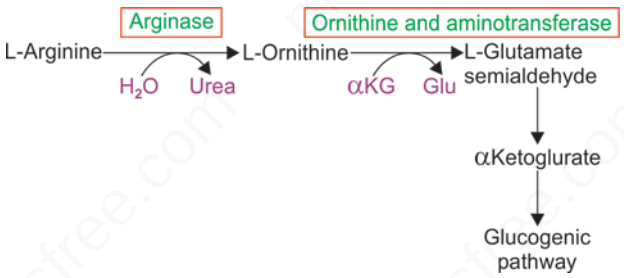


Fig. 1.35: Metabolism of arginine

Arginine

- Glucogenic
- Semiessential Amino Acid
- L Glutamate Semi aldehyde to α Keto Glutarate to Glucogenic pathway.

Metabolic Functions of Arginine

- Nitric Oxide Synthesis
- Agmantine
- Arginine splits to Ornithine and Urea. (Terminal step in Urea Cycle)
- Creatine.

Nitric Oxide

- Uncharged molecule having an unpaired electron, so it is highly reactive, free radical
- Very short half-life (0.1 seconds)
- Formerly called Endothelium Derived Relaxing Factor
- Gaseous molecule
- Second messenger is cGMP.

Functions of Nitric Oxide

- Potent Vasodilator
- Involved in Penile erction
- Neurotransmitter in brain and Peripheral Nervous System
- Low level of NO involved in Pylorospasm in Congenital Hypertrophic Pyloric Stenosis
- Inhibit adhesion, activation and aggregation of Platelets.

Therapeutic uses of Nitric Oxide

- Inhalation of Nitric Oxide in the treatment of Pulmonary Hypertension
- Treatment of Impotence (Sildenafil inhibit cGMP Phosphodiesterase)
- Glyceryl Nitrite which is converted to Nitric Oxide is used in Angina Pectoris.

Synthesis of Nitric Oxide

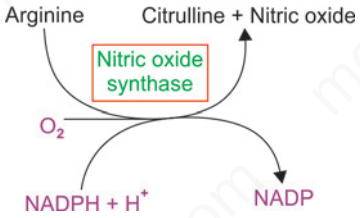


Fig. 1.36: Synthesis of nitric oxide

Nitric Oxide Synthase

- Cytosolic Enzyme
- Mono oxygenase

Five Redox Cofactors are:

1. NADPH
2. FAD
3. FMN
4. Heme
5. Tetrahydrobiopterin

Three Major Isoforms of Nitric Oxide Synthase

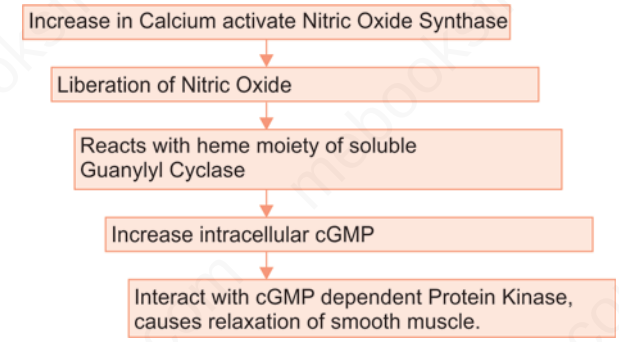
Subtype	Name	Characteristics	Deficiency leads to
1.	nNOS	First identified in the **neurons** Activated by increase in Ca^{2+}	Pyloric Stenosis Aggressive Sexual Behavior

Contd...

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Subtype	Name	Characteristics	Deficiency leads to
2.	iNOS	Prominent in **macrophages** Independent of elevated Ca^{2+}	More susceptible to certain types of infection
3.	eNOS	First identified in endothelial cells. Activated by Ca^{2+}	Elevated mean blood pressure.

Mechanism of Action of Nitric Oxide



Agmatine

- Derived from Arginine by decarboxylation
- Properties of Neurotransmitter
- May have Antihypertensive Properties.

HISTIDINE

- Semi essential amino acid
- Contains Imidazole ring
- Maximum buffering capacity at physiological pH.

Metabolism of Histidine

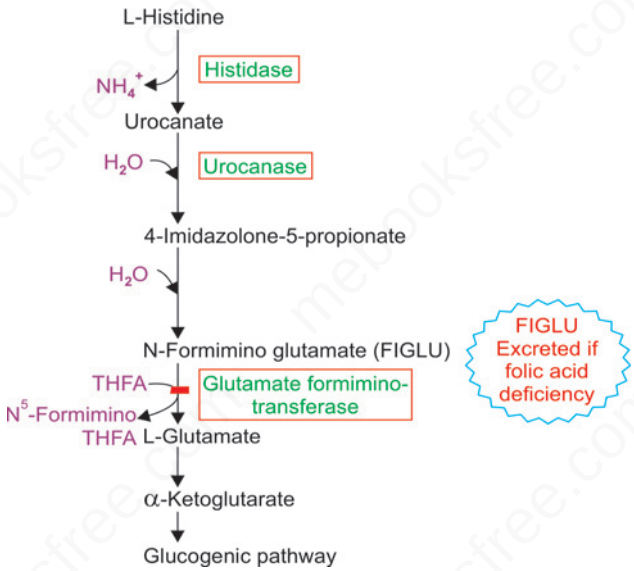


Fig. 1.37: Metabolism of histidine

Important points of the histidine metabolism pathway

- Urocanate is a derivative of Histidine
- FIGLU is Formimino Glutamic Acid
- FIGLU is derived from Histidine
- In Folic Acid deficiency FIGLU is excreted in Urine.

Histidine Load Test

- To identify Folic Acid deficiency.
- FIGLU excreted in urine is measured following a Histidine load.

Biologically important compounds derived from Histidine

- Histamine from histidine by decarboxylation. PLP is a coenzyme
- Carnosine (Beta Alanyl Histidine)
- Anserine (Methyl Carnosine)
- Homocarnosine (Gamma Amino Butyryl Histidine)
- Ergothionine

Function of Histamine and receptor responsible for its action

Type of receptor	Effect
H1	Smooth muscle contraction. Increased vascular permeability.
H2	Gastric HCl secretion.
H3	Synthesis and release of histamine in the brain.

- Metabolic error due to deficiency of Histidase is histidinemia.

ACIDIC AMINO ACIDS

Glutamic Acid (Glutamate)

- Nonessential Amino Acid
- Glucogenic Amino Acid
- Central role in metabolism of Amino Acid
- Amino group of all Amino Acids are concentrated as **Glutamate**Q by transamination.

Biosynthesis of Glutamate

By reductive amidation of α Ketoglutarate catalyzed by Glutamate dehydrogenase.

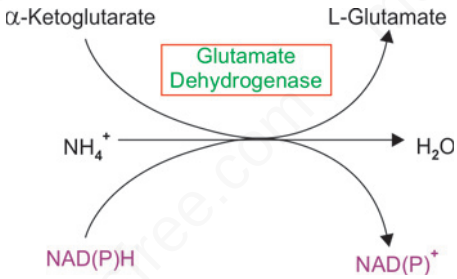


Fig. 1.38: Biosynthesis of glutamate

Metabolic Functions of Glutamic Acid

- **Synthesis of N-Acetyl Glutamate**
  Positive regulator of Carbamoyl Phosphate synthetase-1 of urea cycle
  Glutamic Acid + Acetyl CoA ⟶ N-Acetyl Glutamate + CoASH
- **Synthesis of Glutathoine (Gamma Glutamyl Cysteinyl Glycine)**
- **Synthesis of Gamma Amino Butyric Acid (GABA)**
  – Glutamic Acid on decarboxylation gives GABA.
  – PLP is the coenzyme.

Glutamine

Biosynthesis of Glutamine

Glutamine synthesized from Glutamic Acid by Glutamine Synthetase.

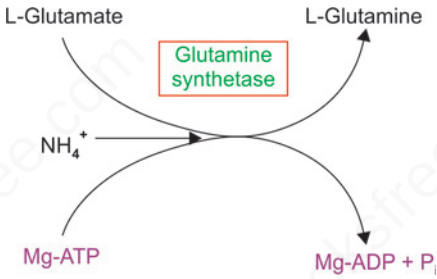


Fig. 1.39: Biosynthesis of glutamine

Metabolic functions of glutamine

- Converts inorganic ammonium ions into the α amino nitrogen of amino acid. This reaction is called first line trapping of Ammonia
- Carry amino group from Brain and most other tissues
- N3 and N9 of Purine ring derived from Glutamine.
- N3 of Pyrimidine is derived from glutamine.
- Source of NH_2 group of Guanine and Cytosine
- Glutamine is a conjugating agent
- Source of Ammonia excretion for Kidney, which has a role in renal regulation of acid base balance.

Aspartic Acid (Aspartate)

- Nonesssential
- Glucogenic amino acid.

Synthesis of Aspartate

Transamination of Oxaloacetate forms Aspartate.

Functions of Aspartate

- Contribute its alpha amino group for Urea Synthesis.
- Contributes to Purine Synthesis
- Contributes to Pyrimidine Synthesis.

Canavan Disease

- Autosomal recessive disorder
- More prevalent in individuals of Ashkenazi Jewish descent than in other ethnic groups.

Biochemical defect

- Deficiency of aspartoacylase, leads to Canavan disease
- *N*-Acetylaspartic acid, a derivative of aspartic acid, is synthesized in the brain
- The exact function of *N*-acetylaspartic acid is unknown, but it may serve as a reservoir for acetate, which is needed for myelin synthesis
- Aspartoacylase, cleaves the *N*-acetyl group from *N*-acetylaspartic acid.

Characterized by

- **Leukodystrophy**
- Excessive excretion of *N*-acetylaspartic acid.

Diagnosis

- Aspartoacylase deficiency can be determined in skin fibroblasts
- Increased excretion of *N*-acetylaspartic acid in the urine.

Asparagine

Synthesis of Asparagine

Aspartate is converted to Asparagine by Asparagine Synthetase.

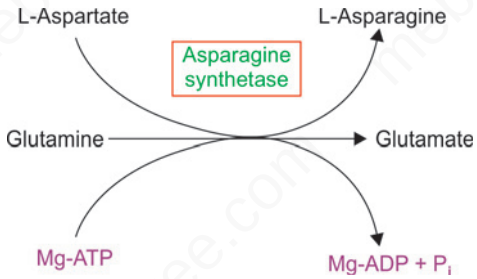


Fig. 1.40: Biosynthesis of asparagine

Asparagine Synthetase

- Asparagine Synthetase is analogous to Glutamine Synthetase
- In Asparagine Synthetase, Glutamine rather than ammonium ions, provides nitrogen
- Hence cannot fix ammonia like Glutamine Synthetase..
- Bacterial Asparagine Synthetase can however, also use ammonium ion.

Catabolism of Glutamate, Glutamine, Aspartate and Asparagine

- Glutamine and Glutamate forms Alpha Keto Glutarate[9]
- Asparagine and Aspartate forms Oxaloacetate.[9]

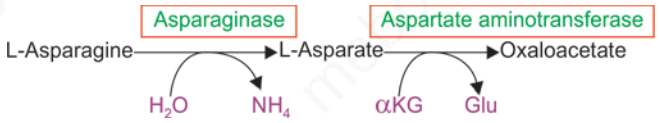


Fig. 1.41: Catabolism of asparagine

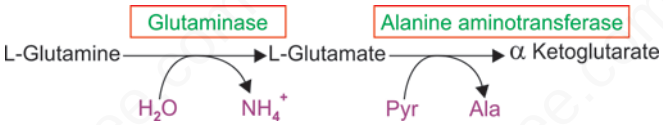


Fig. 1.42: Catabolism of glutamine

AMINO ACIDS ENTER INTO TCA CYCLE AT DIFFERENT LEVELS

To Alpha Ketoglutarate

Arginine, Histidine, Glutamine, Proline to Glutamate. This is transaminated to Alpha Ketoglutarate.

To Succinyl CoA[Q]

- Valine
- Isoleucine
- Methionine
- Threonine
- *Remember VIM to Succinyl CoA.*

To Fumarate[Q]

- Tyrosine
- Phenylalanine
- Aspartate.

To Oxaloacetate[Q]

Asparagine to Aspartate, which is transaminated to Oxaloacetate.

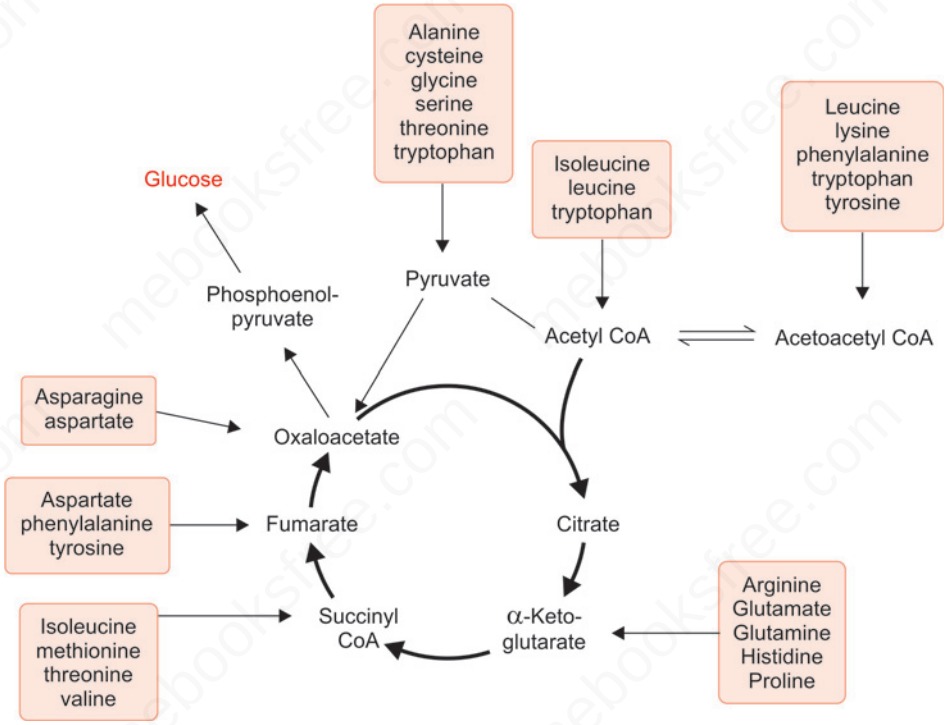


Fig. 1.43: Entry of amino acids to TCA cycle

QUICK REVIEW POINTS FOR NATIONAL BOARD PATTERN OF EXAMS

- Amino acid that absorbs UV light-Tryptophan, Phenylalanine,Tyrosine
- Amino acid with no asymmetric carbon atom-Glycine
- Beta Alanine is derived from Uracil and Cytosine
- Amino acid at isoelectric pH has no net charge
- Most common amino acid that undergo oxidative deamination is Glutamate
- Coenzyme for transamination reaction is Pyridoxal Phosphate (PLP)
- Amino acid that transport ammonia from most organs including the brain is Glutamine

- Amino acid that transport ammonia from skeletal muscle is Alanine
- The nitrogen atoms of Urea are contributed by Ammonia and Aspartate
- The rate limiting step of Urea Cycle is Carbamoyl Phosphate Synthetase I
- Most common urea cycle disorder is Hyperammonemia Type II (Ornithine Transcarbamoylase Defect)
- Polyamines are derived from Ornithine, Methionine and Lysine
- Amino acid involved in Cahill Cycle is Alanine
- Amino acid that play an important role during Starvation as a gluconeogenic AA is Alanine
- Transamination concentrate amino group as Glutamate
- Precursor of carnitine is Lysine and Methionine
- Selenocysteine is derived from Serine
- Glutamic acid is decarboxylated to GABA
- Glutamic acid is deaminated to Alpha Ketoglutarate
- Folate trap traps the THFA as its Methyl Derivative.
- Amino acids that enter TCA Cycle as Succinyl Choline is Valine, Isoleucine and Methionine.

Metabolic Disorder and Biochemical Defect

Metabolic disorder	Biochemical defect
Hyperammonemia Type I	Carbamoyl Phosphate Synthetase I
Hyperammonemia Type II	Ornithine Transcarbamoylase
Citrullinemia Type I	Arginino Succinate Synthetase
Citrullinemia Type II	Citrin (Aspartate-Glutamate) Transporter
Arginino Succinic Aciduria	Arginino Succinate Lyase
Argininemia	Argininase
HHH syndrome	ORNT-I defect (Ornithine Permease)
Classic Phenyl Ketonuria	Phenylalanine Hydroxylase
Alkaptonuria	Homogentisate Oxidase
Tyrosinemia Type I	Fumarylaceto Acetate Hydrolase
Tyrosinemia Type II	Tyrosine Transaminase
Tyrosinemia Type III	Para Hydroxyphenylpyruvate hydroxylase/ Para hydroxyl Phenylpyruvate-Dioxygenase
Hawkinsinuria	Para Hydroxyphenylpyruvate hydroxylase/ Para hydroxylphenylpyruvate Dioxygenase is mutant, so that it catalyzes only partial reaction.
Segawa Syndrome	GTP Cyclohydrolase
Albinism	Tyrosinase

Contd...

Contd...

Metabolic disorder	Biochemical defect
Pheochromocytoma	Excess production of Catecholamines
Carcinoid Syndrome	Excess production of Serotonin
Hartnup's Disease	Defective absorption of Tryptophan and other neutral amino acids from renal tubules and intestines
Primary Hyperoxaluria Type I	Alanine–Glyoxylate Aminotransferase
Primary Hyperoxaluria Type II	D–Glycerate Dehydrogenase/Glyoxylate reductase Enzyme Complex
Nonketotic Hyperglycinemia	Glycine Cleavage System
Classic Homocystinuria	Cystathionine Beta Synthase
Nonclassic Homocystinuria	I. Methylcobalamin formation defect II. Methylene THFA Reductase
Cystathioninuria	Cystathionase
Cystinuria	Defective reabsorption of Cystine, Ornithine, Lysine and Arginine
Oasthouse Syndrome	Malabsorption of Methionine and other neutral amino acids
Type IA MSUD	$E_{1\alpha}$ gene that codes for Branched Chain Keto acid Decarboxylase component of Branched Chain Ketoacid Dehydrogenase Complex
Type IB MSUD	$E_{1\beta}$ gene that codes for Branched Chain Keto acid Decarboxylase component of Branched Chain Ketoacid Dehydrogenase Complex
Type II MSUD	E2 gene that codes for Dihydrolipoyl Transacylase component of Branched Chain Ketoacid Dehydrogenase Complex
Type IV MSUD	E1α gene that codes Dihydrolipomide Dehydrogenase component of Branched Chain Keto acid Dehydrogenase Complex
Isovaleric Aciduria	Isovaleryl CoA Dehydrogenase
Canavan Disease	N Asparto Acylase

Specialized Products from Amino Acids

Amino acid	Metabolic products
Tyrosine	• Melanin • Catecholamines (Epinephrine, Norepinephrine, Dopamine) • Thyroxine
Tryptophan	• Serotonin • Melatonin • Niacin
Cysteine	• Cystine • Taurine • Glutathione • Betamercaptoethanolamine
Glycine	• Purine • Heme • Glutathione • Creatinine

Contd...

Contd...

Amino acid	Metabolic products
Arginine	• Nitric oxide • Arginine • Arginine splits to Ornithine and Urea • Creatine
Histidine	• FIGLU • Histamine
Glutamate	• N acetyl Glutamate Glutathione • Gamma Amino Butyric Acid
Glutamine	• N3 and N9 of Purine • N3 of Pyrimidine
Aspartate	• Purine • Pyrimidine • Urea Synthesis

Peculiar Odors in Different Amino Acidurias

Inborn error of Metabolism	Urine odor
Glutaric acidemia (type II)	**Sweaty feet, acrid**
Hawkinsinuria	**Swimming pool**
Isovaleric Acidemia	**Sweaty feet, acrid**
3-Hydroxy-3- methylglutaric aciduria	**Cat urine**
Maple syrup urine disease	**Maple syrup**
Hypermethioninemia	**Boiled cabbage**
Multiple carboxylase deficiency	**Tomcat urine**
Oasthouse urine disease	**Hops-like**
Phenylketonuria	**Mousey or musty**
Trimethylaminuria	**Rotting fish**
Tyrosinemia	**Boiled cabbage, rancid butter**

REVIEW QUESTIONS

CLASSIFICATION OF AMINO ACIDS

1. Selenocysteine is coded by: **(AIIMS Nov 2015)**

a. UAG
b. UGA
c. UAA
d. GUA

Ans. b. UGA

- Stop codon UGA codes Selenocysteine
- Stop codon UAG codes Pyrrolysine

2. All of the following are essential amino acids except: **(AIIMS May 2006)**

a. Methionine
b. Lysine
c. Alanine
d. Leucine

Ans. c. Alanine

(Ref: Harper 30/e p282, Table 27-1)

Based on nutritional requirement amino acids classified into:

- *Essential:* Those amino acids which cannot be synthesized in the body. Hence these amino acids are to be supplied in the diet.
- *Semiessential:* Growing children require them in the food, but not essential in adults.
- *Nonessential:* Amino acids which can be synthesized in the body, hence not required in the diet.

Essential (MettVilPhly Read As Met Will Fly)	All the other amino acid	Nonessential
Methionine	Arginine	All the other amino acid
Threonine		
Tryptophan		
Valine		
Isoleucine		
Leucine		
Phenylalanine		
Lysine		

3. Polar amino acids is/are: **(PGI May 2012)**

a. Serine
b. Tryptophan
c. Tyrosine
d. Valine
e. Lysine

Ans. a., e. Serine, Lysine

(Ref: Harper 30/e p18, Table 3-2)

Classification of amino acids based on side chain characteristics (polarity)

- **Polar Amino Acids (Hydrophilic):**
  - Uncharged amino acids are Serine, Threonine, Glutamine, Asparagine, Cysteine, Glycine Charged Amino Acids are Aspartic Acid, Glutamic Acid, Histidine, Arginine, Lysine.
- **Nonpolar Amino Acid (Hydrophobic)**
  - Alanine, Leucine, Isoleucine, Valine, Phenyl Alanine, Tyrosine, Tryptophan, Proline, Methionine.

4. Nonpolar Amino acids are: **(PGI Nov 2010)**
a. Alanine
b. Tryptophan
c. Isoleucine
d. Lysine
e. Tyrosine

Ans. a, b, c, e *(Ref: Harper 30/e p18 table 3-2)*

5. Hydrophobic amino acids are: **(PGI May 2010)**
a. Methionine
b. Isoleucine
c. Tyrosine
d. Alanine
e. Asparagine

Ans. a, b, c, d *(Ref: Harper 30/e p18, table 3-2)*

6. Basic amino acids is/are:
a. Leucine
b. Arginine
c. Lysine
d. Histidine

Ans. b, c, d *(Ref: Harper 30/e p17, Table 3-1)*
- Basic amino acids are Histidine, Arginine and Lysine
- Acidic amino acids are Aspartic Acid (Aspartate), Glutamic Acid (Glutamate)

7. Guanidinium group is associated with:
a. Tyrosine **(PGI June 2009)**
b. Arginine
c. Histidine
d. Lysine
e. Tryptophan

Ans. b. Arginine

(Ref: Harper 30/e page 19)

Special Groups Present in Amino Acids

Amino acid	Special group
Arginine	Guanidinium[a]
Phenylalanine	Benzene
Tyrosine	Phenol
Histidine	Imidazole[a]
Proline	Pyrrolidine
Methionine	Thioether Linkage
Tryptophan	Indole
Cysteine	Thioalcohol (SH)

8. Amino acid produced by adding hydroxyl group to benzene ring chain of phenylalanine:
a. Threonine **(Kerala 2011)**
b. Histidine
c. Tyrosine
d. Serine

Ans. c. Tyrosine *(Ref: Harper 30/e page 19 table 3-2)*
- Special group in phenylalanine is benzene ring
- Special group in Tyrosine is Phenol ring
- The enzyme that hydroxylate Phenylalanine to Tyrosine is Phenylalanine Hydroxylase

9. Sulfur containing amino acid is: **(Ker 2009)**
a. Cysteine
b. Leucine
c. Arginine
d. Threonine

Ans. a. Cysteine
- Sulur containing amino acids are Cysteine and Methionine.
- The Sulphur of Cysteine is provided by Methionine.
- Special group in Cysteine is Sulfhydryl group (Thioalcohol (-SH)
- Special group in Methionine is Thioalcohol (C-S-C)

10. Which of the following is a nonaromatic amino acid with a hydroxyl R-group? **(Kerala 2012)**
a. Phenylalanine
b. Lysine
c. Threonine
d. Methionine

Ans. c. Threonine *(Ref: Harper 30/e p17, Table 3.1)*
- Aromatic amino acid with hydroxyl group-Tyrosine
- Nonaromatic amino acid with hydroxyl group are Serine and Threonine

11. Which is not an essential amino acid?
a. Tryptophan **(Kerala 2006)**
b. Threonine
c. Histidine
d. Cysteine

Ans. d. Cysteine *(Ref: Harper 30/e p282)*

Essential Amino acids are Methionine, Threonine, Tryptophan, Valine, Isoleucine, Leucine, Phenylalanine, Lysine (Mnemonic MeTT VIL PhLy) and Histidine

Semiessential Amino acids is Arginine.

12. Which of the following is not an aromatic amino acid?
a. Phenylalanine
b. Tyrosine
c. Tryptophan
d. Valine

Ans. d. Valine *(Ref: Harper 30/e p17, Table 3-1)*

Aromatic amino acids are
- Histidine (with Imidazole ring)
- Phenyl Alanine (Benzene ring)
- Tyrosine (Phenol ring)
- Tryptophan (Indole ring)

13. Which of the following side chains is least polar? (AI 2009)
a. Methyl
b. Carboxyl
c. Amino
d. Phosphate

Ans. a. Methyl

14. Which of the following group contains only nonessential amino acid? (NBE pattern Q)
a. Acidic Amino Acid
b. Basic Amino Acid
c. Aromatic Amino Acid
d. Branched chain amino Acid

Ans. a. Acidic Amino acid *(Ref: Harper 30/e p282)*
- Group of amino acid that contain only essential amino acid is **Branched chain amino acids (Leucine, Isoleucine, Valine)**
- Group of amino acid that contain only nonessential amino acid is **Acidic Amino acids, Amide group containing amino acids, Imino acid, Simple amino acids**

15. Amide group containing amino acid is: (NBE Pattern Q)
a. Glutamate
b. Glutamic acid
c. Glutamine
d. Aspartate

Ans. c. Glutamine
- Glutamine and Asparagine are Amide group containing amino acids.
- Aspartate and Glutamate are Acidic amino acid.

16. Semiessential amino acids are: (PGI 94)
a. Arginine
b. Histidine
c. Glycine
d. Phenylalanine

Ans. a. Arginine *(Ref: Harper 30/e p282, Table 27-1)*

Semiessential Amino acid-Arginine

17. Aminoacyl t-RNA is required for all except: (AI 2000)
a. Hydroxyproline
b. Methionine
c. Cysteine
d. Lysine

Ans. a. Hydroxyproline
- Derived amino acids do not require Aminoacyl tRNA.
- Among the above options Hydroxyproline is a derived amino acid

Derived Amino Acid Seen in Protein[Q]

4-Hydroxy Proline	• Found in Collagen
5-Hydroxy Lysine	• Vitamin C is needed for hydroxylation.
Methyl lysine	• Found in Myosin
Gamma carboxy glutamate	• Found in clotting factors, like Prothrombin that bind Ca^{2+} • Vitamin K is needed for Gamma carboxylation.
Cystine	• Found in proteins with disulphide bond.[Q 2014 DNB] • Two cysteine molecules join to form cystine e.g., Insulin, Immunoglobulin
Desmosine	• Found in Elastin[Q AIIMS Nov 2014]

Derived Amino Acid Not seen in Protein[Q]

Ornithine	Intermediates of Urea Cycle
Arginosuccinate	
Citrulline	
Homocysteine	Derived from Methionine[Q]
Homoserine	Product of Cysteine Biosynthesis
Glutamate-γ semialdehyde	Serine Catabolite

Properties of Amino Acids

18. Replacing alanine by which amino acid will increase UV absorbance of protein at 280 nm wavelength: (AIIMS Nov 2008)
a. Leucine
b. Proline
c. Arginine
d. Tryptophan

Ans. d. Tryptophan *(Ref: Harper 30/e p21, 22)*

Amino Acid Absorb UV Light

Amino Acids which absorb 250–290 nm (Maximum at 280 nm) UV light are tryptophan, phenylalanine, tyrosine.

Maximum absorption of UV light by tryptophan.

Remember
Aromatic amino acids absorb UV light

19. Which of the following proteins cannot be phosphorylated using Protein kinase in prokaryotic organisms? (AI 2012)
a. Threonine
b. Tyrosine
c. Serine
d. Asparagine

Ans. d. Asparagine *(Ref: Harper 30/e p93, Chapter 9)*

Protein kinases phosphorylate proteins by catalyzing transfer of the terminal phosphoryl group of ATP to the hydroxyl groups of seryl, threonyl, or tyrosyl residues, forming O-phosphoseryl, O-phosphothreonyl, or O-phosphotyrosyl residues, respectively

- Commonest site of phosphorylation is Serine and Threonine followed by Tyrosine.

20. Carboxylation of clotting factors by vitamin K is required to be biologically active. Which of the following amino acid is carboxylated? (AIIMS Nov 2008)

a. Histidine
b. Histamine
c. Glutamate
d. Aspartate

Ans. c. Glutamate *(Ref: Harper 30/e p717)*

- The Vitamin that act as coenzyme for carboxylation is Biotin
- The vitamin that act as coenzyme for gamma carboxylation is Vitamin K
- The Proteins that are gamma carboxylated by Vitamin K are:
  - Factor II (Prothrombin),
  - Factor VII (Proconvertin or Serum Prothrombin conversion Accelerator, SPCA),
  - Factor IX (Antihemophilic factor or Christmas factor),
  - Factor X (Stuart Prower factor),
  - Protein C, Protein S,
  - Osteocalcin, Nephrocalcin
  - Product of gene gas 6

21. Which of the following is/are not optically inactive amino acids? (PGI May 2014)

a. Threonine
b. Tyrosine
c. Valine
d. Glycine
e. Serine

Ans. a, b, c, e. *(Ref: Harper 30/e p19)*

- Glycine is the only optically inactive amino acid

22. Property of photochromisity is seen amongst the following amino acids: (AI 1997)

a. Unsaturated amino acid
b. Aromatic amino acid
c. Monocarboxylic acid
d. Dicarboxylic acid

Ans. b. Aromatic amino acid

(Ref: Harper 30/e p21, 22)

Amino Acid Absorb UV Light

Amino Acids which absorb 250–290 nm (Maximum at 280 nm) UV light are tryptophan, phenylalanine, tyrosine. Maximum absorption of UV light by tryptophan.

Remember
Aromatic amino acids absorb UV light

23. The property of proteins to absorb ultraviolet rays of light is due to: (AIIMS June 99)

a. Peptide bond
b. Imino group
c. Disulfide bond
d. Aromatic amino acid

Ans. d. Aromatic amino acid

(Ref: Harper 30/e p21, 22)

24. All biologically active amino acids are:

a. L-forms
b. D-forms
c. Mostly D-forms
d. D- and L- forms

Ans. a. L forms *(Refe: Harper 30/e p18)*

- **Amino acids mostly exists in L forms**
- **Carbohydrates exists in D forms**

25. Optically inactive amino acid is: (AI 99)

a. Proline
b. Glycine
c. Lysine
d. Leucine

Ans. b. Glycine

- Only optically inactive amino acid is Glycine

26. Flexibility of protein depends on: (AI 1994)

a. Glycine
b. Tryptophan
c. Phenylalanine
d. Histidine

Ans. a. Glycine *(Ref: Harper 30/e p39)*

- Glycine having the smallest R group fit in to small spaces and induces bends in the alpha helix.
- Glycine is usually present in beta turns.

27. Which amino acid can protonate and deprotonate at neutral pH? (AIIMS May 95)

a. Histidine
b. Leucine
c. Glycine
d. Arginine

Ans. a. Histidine

- Amino acid which can protonate and deprotonate means those which can act as buffer.
- Amino acid whose pKa = pH of the medium has maximum buffering capacity.
- pKa of imidazole group of histidine is 6.5–7.4.
- At pH = 7, Imidazole group of histidine can act as buffer.

28. Phosphorylation of amino acid by: (PGI June 98)

a. Serine
b. Tyrosine
c. Leucine
d. Tryptophan

Ans. a. Serine, **b.** Tyrosine

(Ref: Harper 30/e p93, Chapter 9)

29. Which of the following amino acid is purely Glucogenic? (NBE Pattern Q)

a. Valine
b. Lysine
c. Alanine
d. Glycine

Ans. c. Alanine > Valine/Glycine

- Lysine is both ketogenic and gluco(glyco)genic
- Glycine, Alanine and Valine are purely Glucogenic.
- But Alanine is the principal Glucogenic amino acid.

Remember
Glucose Alanine cycle in starvation for provision of substrate for gluconeogenesis.

GENERAL AMINO ACID METABOLISM

Digestion and Absorption of Proteins, Transamination and Transport of Amino Acids

30. Increased alanine during prolonged fasting represents: (AIIMS Nov 2011)

a. Increased breakdown of muscle proteins
b. Impaired renal function
c. Decreased utilization of amino acid from Gluconeogenesis
d. Leakage of amino acids from cells due to plasma membrane damage

Ans. a. Increased break down of muscle protein

During prolonged fasting, there is increased gluconeogenesis. Alanine provided by muscle is one of the substrates for gluconeogenesis.

This is called Glucose Alanine Cycle or Cahill Cycle.

So plasma level of Alanine rises in prolonged starvation.

Remember
- In prolonged fasting plasma level of Alanine rises.
- In hyperammonemia plasma level of Glutamine rises.

31. Transfer of an amino group from an amino acid to an alpha keto acid is done by: (AI 2011)

a. Transaminases
b. Aminases
c. Transketolases
d. Deaminases

Ans. a. Transaminases *(Ref: Harper 30/e p290)*

Keypoints transamination

- Interconvert pair of α amino acids and α Ketoacid.
  - Ketoacid formed by transamination from Alanine is Pyruvate
  - Ketoacid formed by transamination from Aspartate is Oxaloacetate.
  - Ketoacid formed by transamination from Glutamate is α Keto Glutarate
- Freely reversible.
- Transmination concentrate α amino group of nitrogen as L-Glutamate.
- L-Glutamate is the only enzyme that undergo significant amount of oxidative deamination in mammals.
- Takes place via ping pong mechanism.
- Takes an important role in biosynthesis of nutritionally nonessential amino acids.
- Specific for one pair of substrate but nonspecific for other pair of substrates.
- Pyridoxal Phosphate is the coenzyme

32. The amino acid which serves as a carrier of ammonia from skeletal muscle to liver is: (AI 2006)

a. Alanine
b. Methionine
c. Arginine
d. Glutamine

Ans. a. Alanine *(Ref: Lippincott 6/e p253)*

- **Transport form of Ammonia from most tissues including brain is Glutamine**
- **Transport form of Ammonia from skeletal muscle is Alanine.**

33. Glutamine in blood acts as: (PGI Dec 98)

a. NH_3 transporter
b. Toxic element
c. Stored energy
d. Abnormal metabolite

Ans. a. Ammonia transporter

Transport form of ammonia from brain and most other tissues

34. Amino acid absorption is by: (NBE Pattern qn)

a. Facilitated transport
b. Passive transport
c. Active transport
d. Pinocytosis

Ans. c. Active Transport *(Ref: Harper 30/e p539)*

Free amino acids are absorbed across the intestinal mucosa by **sodium-dependent active transport**. There are several different amino acid transporters, with specificity for the nature of the amino acid side-chain.

Transporters of Amino Acids

- For Neutral Amino Acids
- For Basic Amino acids and Cysteine
- For Imino Acids and Glycine
- For Acidic Amino Acids
- For Beta Amino Acids (Beta Alanine)

Meisters Cycle

- For absorption of Neutral Amino acids from Intestines, Kidney tubules and brain.
- The main role is played by Glutathione. (GSH)
- For transport of 1 amino acid and regeneration of GSH 3 ATPs are required.

Disorders associated with Meister's Cycle Oxoprolinuria

- **5 Oxoprolinase** deficiency leads to Oxoprolinuria

Disorders Associated with Absorption of Amino acids

Hartnup's Disease	Malabsorption of neutral amino acids, including the essential amino acid tryptophan
	SLC6A19, which is the major luminal sodium-dependent neutral amino acid transporter of small intestine and renal tubules, has been identified as the defective protein
Blue Diaper Syndrome or Drummond Syndrome Indicanuria	Tryptophan is specifically malabsorbed and the defect is expressed only in the intestine and not in the kidney. Intestinal bacteria convert the unabsorbed tryptophan to indican, which is responsible for the bluish discoloration of the urine after its hydrolysis and oxidation
Cystinuria	Dibasic amino acids, including **cystine, ornithine, lysine, and arginine** are taken up by the Na-independent SLC3A1/ SLC7A9, in the apical membrane which is defective in cystinuria **Most common disorder associated with Amino acid malabsorption.**

Contd...

Contd...

Lysinuric Protein Intolerance	(SLC7A7) carrier at the basolateral membrane of the intestinal and renal epithelium is affected, with failure to deliver cytosolic dibasic cationic amino acids into the paracellular space in exchange for Na^+ and neutral amino acids.
Oasthouse Urine Disease **(Smith Strang Disease)**	A methionine-preferring transporter in the small intestine was suggested to be affected. Cabbage-like odor, containing 2-hydroxybutyric acid, valine, and leucine.
Iminoglycinuria	Malabsorption of proline, hydroxyproline, and glycine due to the proton amino acid transporter SLC36A2 defect
Dicarboxylic Aciduria	Excitatory amino acid carrier SLC1A1 is affected. Associated with neurologic symptoms such as POLIP (polyneuropathy, ophthalmoplegia, leukoencephalopathy, intestinal pseudo-obstruction

35. Nontoxic form of storage and transportation of ammonia: (NBE Pattern Q)

a. Aspartic acid
b. Glutamic acid
c. Glutamine
d. Glutamate

Ans. c. Glutamine

- Transport form of Ammonia from most tissues is Glutamine.
- The enzyme responsible is called Glutamine Synthetase.
- Belong to Ligase class.
- Require ATP.

Urea Cycle

36. Urea cycle enzymes are: (PGI May 2010)

a. Glutaminase
b. Asparginase
c. Argininosuccinate synthetase
d. Ornithine transcarbamoylase
e. Glutamate dehydrogenase

Ans. c. Argininosuccinate synthetase, **d.** Ornithine transcarbamoylase *(Ref: Harper 30/e p293)*

Reactions of Urea Cycle

The first two reaction takes place in the mitochondria. The rest of the reactions takes place in the cytoplasm.

Carbamoyl Phosphate Synthetase–I (CPS-I)

- Carbamoyl Phosphate is formed from the condensation of CO_2, Ammonia and ATP.
- CPS-I is the rate limiting (pacemaker) enzyme in this pathway

- CPS-I is active only in the presence of N-Acetyl Glutamate, an allosteric activator.
- This step require 2 mols of ATPs.

Ornithine Transcarbamoylase (OTC)

Transfer carbamoyl group of Carbamoyl Phosphate to Ornithine forming Citrulline. Subsequent steps takes place in the cytoplasm.

Argininosuccinate Synthetase

- Links Amino nitrogen of Aspartate to Citrulline and provides second nitrogen of Urea
- This reaction requires ATP.
- Two inorganic phosphates are utilized.

Argininosuccinate Lyase

Cleavage of Arginiosuccinate to Arginine and Fumarate.

Arginase[Q]

Hydrolytic cleavage of Arginine, releases Urea and reforms Ornithine which reenter into mitochondria

Remember
All the enzymes in the Cytoplasm starts with the letter 'A'

37. Which enzymes are part of urea cycle? **(PGI 2012)**

a. Ornithine Transcarbamoylase
b. Asparaginase
c. Glutamate Synthase
d. Arginosuccinase.

Ans. a. Ornithine Transcarbamoylase, **d.** Arginosuccinase *(Ref: Harper 30/e p293)*

Enzymes of urea cycle and its classes

Enzymes name	Class of enzyme it belongs
Carbamoyl-phosphate synthase I	Class 6 (Ligase)
Ornithine carbamoyl transferase	Class 2 (Transferase)
Argininosuccinate synthase	Class 6 (Ligase)
Argininosuccinate lyase (Arginino-Succinase)	Class 4 (Lyase)
Arginase	Class 3 (Hydrolase)

38. Urea cycle occurs in: **(AI 2011)**

a. Liver
b. GIT
c. Spleen
d. Kidney

Ans. a. Liver

- Site of urea synthesis in liver mitochondria and cytosol.
- Derived amino acids which has almost exclusive role in urea cycle are Ornithine, Citrulline, Arginino-succinate.
- Four amino acids which has no net loss or gain in urea cycle is ornithine, citrulline, arginino succinate, arginine.

39. In which of the following condition there is increased level of ammonia in blood? **(Ker 2008)**

a. Ornithine transcarbamoylase deficiency
b. Galactosemia
c. Histidinemia
d. Phenyl ketonuria

Ans. a. Ornitine transcarbamoylase

Increased ammonia in blood is suggestive of a urea cycle disorder. So answer is an enzyme of urea cycle.

Hyperammonemia Type II (OTC Deficiency)

- Most common Urea Cycle disorder[Q]
- Disorder with **X-linked partially dominant inheritance** (All other Urea Cycle Disorders are Autosomal Recessive)
- Urea cycle disorder with Orotic Aciduria
- Marked elevations of plasma concentrations of glutamine and alanine with low levels of citrulline and arginine
- Orotate may precipitate in urine as a pink colored gravel or stones.

40. Urea cycle occurs in: **(Ker 2008)**

a. Cytoplasm
b. Mitochondria
c. Both
d. Endoplasmic reticulum

Ans. c. Both

The pathways that takes place in two compartments are:

- Heme Synthesis
- Urea Cycle
- Gluconeogenesis

41. Which of the following enzymes(s) is/are not involved in Urea Cycle? **(PGI May 2012)**

a. Glutamate Dehydrogenase
b. Argininosuccinate Synthetase
c. α Ketoglutarate Dehydrogenase
d. Isocitrate Dehydrogenase
e. Fumarase

Ans. a, c, d, e *(Ref: Harper 30/e p276, 277)*

- Glutamate Dehydrogenase-Oxidative deamination
- Argininosuccinate Synthetase-Urea Cycle
- Alpha Keto Glutarate Dehydrogenase and Isocitrate Dehydrogenase-TCA Cycle
- Fumarase-TCA Cycle

42. Glutamate dehydrogenase in mitochondria is activated by: **(NBE pattern Q)**

a. ATP
b. GTP
c. NADH
d. ADP

Ans. d. ADP *(Ref: Harper 30/e p291)*

- Glutamate Dehydrogenase (GDH)
- Liver Glutamate Dehydrogenase (GDH) is allosterically inhibited by ATP, GTP, NADH.
- Liver Glutamate Dehydrogenase (GDH) is allosterically activated by ADP
- Reversible reaction but strongly favor Glutamate formation
- Can use either NAD^+ or $NADP^+$.

43. Nitrogen atoms of Urea contributed by:

a. Ammonium and Aspartate **(NBE pattern Q)**
b. Ammonium and Glutamate
c. Ammonium and Glycine
d. Ammonium and Asparagine

Ans. a. Ammonium and Aspartate *(Ref: Harper 30/e p293)*

- First nitrogen by Ammonium ion—by the reaction CPS-I
- Second nitrogen by Aspartate—by the reaction Argininosuccinate Synthetase

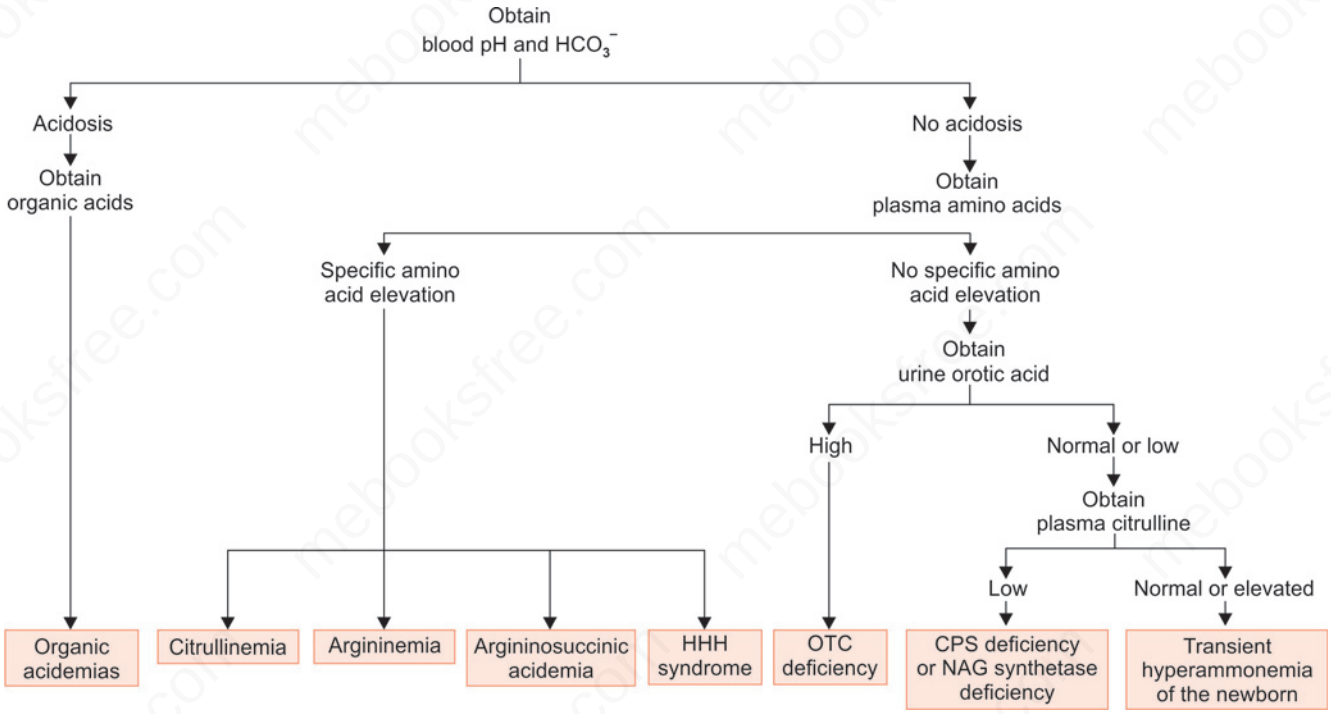
44. A 6-month-old boy admitted with failure to thrive with high glutamine and Uracil in urine Hypoglycemia, high blood ammonia. Treatment given for 2 months. At 8 months again admitted for failure to gain weight. Gastric tube feeding was not tolerated Child became comatose. Parenteral Dextrose given. Child recovered from coma within 24 hours. What is the enzyme defect?

a. CPS1 **(AIIMS May 2015)**
b. Ornitine transcarbamoylase
c. Arginase
d. Argininosuccinate Synthetase

Ans. b. Ornithine transcarbamoylase

(Ref: Nelson: Defects in metabolism of amino acids page 672)

Urea Cycle Disorders



In the given case clue to diagnosis are:

- *High glutamine:* Usually seen in hyperammonemia. Because Ammonia is the transport form of ammonia from brain and most other tissues. So in hyperammonemia Glutamine level is elevated.
- *Increased uracil* in urine can be seen in Ornithine Transcarbamoylase defect because as OTC defective, carbamoyl phosphate in mitochondria spills to cytoplasm. Then it enters into Pyrimidine synthesis. Pyrimidine intermediates and pyrimidines can accumulate. Hence, Uracil in urine.

45. Which of the following is true in relation of urea cycle? (PGI Dec 05)

a. First 2 steps in cytoplasm
b. First 2 steps in mitochondria
c. Defect of enzyme of any step can cause deficiency disease
d. Urea is formed by NH_3, glutamic acid and CO_2
e. Citrulline is formed by combination of carbamoyl phosphate and L. ornithine

Ans. b. First 2 steps in mitochondria, **c.** Defect of enzyme of any step can cause deficiency disease **e.** Citruline is formed by combination of carbomoyl phosphate and L. ornithine

Urea Cycle

- First two steps in mitochondria, rest three steps in the cytoplasm.
- Ornithine condenses with Carbamoyl Phosphate to form citrulline by the action of the enzyme OTC.
- Disorder is associated with all the steps of urea cycle disorders

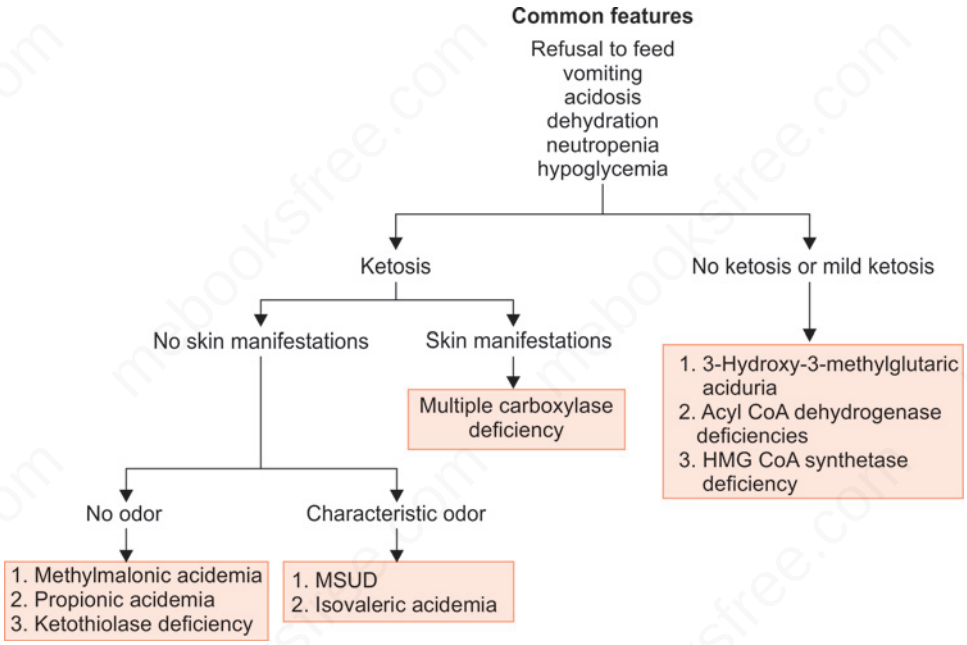
Urea cycle disorders due to enzyme deficiency	
Disorder	**Enzyme defective**
Hyperammonemia Type I	Carbamoyl Phosphate Synthetase I (CPS-I)
Hyperammonemia type -II	Ornithine Transcarbamoylase (OTC)
Citrullinemia Type I (Classic Citrullinemia)	Argininosuccinate synthetase
Arginosuccinic aciduria	Argininosuccinate lyase
Hyperargininemia	Arginase
Urea Cycle Disorders due to Transporter Defect	
Citrullinemia Type II	Citrin (Transport Aspartate and Glutamate) Defect
Hyperammonemia Hyperornithinemia Homocitrullinuria (HHH) Syndrome	Ornithine Transporter Defect

46. A baby presents with refusal to feed, skin lesions, seizures, ketosis, organic acids in urine with normal ammonia; likely diagnosis: (AI 2001)

a. Proprionic aciduria
b. Multiple carboxylase deficiency
c. Maple syrup urine disease
d. Urea cycle enzyme deficiency

Ans. b. Multiple Carboxylase deficiency

(Ref: Nelson: Defects in Amino acid metabolism page 650)



47. True about urea cycle: (PGI May 2015)

a. Nitrogen of the urea comes from alanine and ammonia
b. Uses ATP during conversion of argininosuccinate to arginine
c. On consumption of high amount of protein, excess urea is formed.
d. Occur mainly in cytoplasm
e. Synthesis of argininosuccinate consumes energy

Ans. c. On consumption of high amount of protein, excess urea is formed, **d.** Occur mainly in cytoplasm, **e.** Synthesis of argininosuccinate consumes energy

- Nitrogen of urea comes from Ammonia and Aspartate
- ATP is required for CPS-I and Argininosuccinate Synthetase
- Out of the 5 reactions, 3 reactions occur in cytoplasm. So occur mainly in the cytoplasm.
- On consumption of high protein, urea synthesis is increased.

48. All are true regarding urea cycle except: (PGI Nov 2014)

a. Urea is formed from ammonia
b. Rate limiting enzyme is Ornthine transcarbamoylase
c. Require energy expenditure
d. Malate is a byproduct of urea cycle
e. One nitrogen of urea comes from Aspartate

Ans. a. Urea is formed from ammonia, **c.** Require energy expenditure, **e.** One nitrogen comes from Aspartate

- Nitrogen of urea are contributed by Ammonia and Aspartate.
- 3 ATPs are directly required for urea cycle
- Rate limiting step is Carbamoyl Phosphate Synthase-I
- Fumarate is a byproduct of urea cycle.

49. Enzyme involved in nonoxidative deamination is: (NBE Pattern Q)

a. L-amino acid Oxidase
b. Glutamate Dehydrogenase
c. Glutaminase
d. Amino acid Dehydrases

Ans. d. Amino acid Dehydrases

Some examples of Nonoxidative Deamination[Q NBE pattern]

- **Amino acid Dehydrases** for amino acids with hydroxyl group (Serine, Threonine)
- **Histidase** for histidine
- **Amino acid Desulfhydrases** for amino acids with sulfhydryl group, Cysteine and Homocysteine

50. Which of these is a conservative mutation:

a. Glutamic acid-glutamine (AIIMS Dec 98)
b. Histidine-glycine
c. Alanine-leucine
d. Arginine-aspartic acid

Ans. c. Alanine-leucine

Conservative mutation means an amino acid replaced by another amino acid of same characteristics.

Glutamic acid: Negatively Charged Polar	Glutamine: Uncharged Polar
Histidine: Positively Charged Polar	Glycine: Uncharged Nonpolar
Alanine-Uncharged Nonpolar	**Leucine: Uncharged Nonpolar**
Arginine: Positively Charged Polar	Aspartic Acid: Negatively Charged Polar

INDIVIDUAL AMINO ACID METABOLISM

Aromatic Amino Acids

51. Which is elevated in PLP deficiency?

a. FIGLU (NBE Pattern Q)
b. Xanthurenic acid
c. Methylmalonic acid
d. Homocystine

Ans. b. Xanthurenic acid

- Urinary metabolite in Vitamin B_6 deficiency: Xanthurenic acid
- Urinary Metabolite in Folic Acid Deficiency: Formimino Glutamic acid, Homocystine
- Urinary metabolite in Vitamin B_{12} deficiency: Homocystine, Methyl Malonic Acid

52. Dopamine is synthesized from: (NBE Pattern Q)

a. Tryptophan
b. Threonine
c. Tyrosine
d. Lysine

Ans. c. Tyrosine

Metabolic products formed from Tyrosine are:

- Melanin
- Thyroxine
- Catecholamines (Dopamine, Epinephrine, Norepinephrine)

53. In Phenylketonuria the main aim of first line therapy is: (AIIMS Nov 2010)

a. Replacement of the defective enzyme
b. Replacement of the deficient product
c. Limiting the substrate for deficient enzyme
d. Giving the missing amino acid by diet

Ans. c. Limiting substrate for the deficient enzyme

(Ref: Nelson: Defects in metabolism of Amino acids 20/e page 638)

- The primary goal of therapy is to reduce phenylalanine levels in the plasma and brain.

Treatment of Classical PKU

- *A low–phenylalanine diet*
- Administration of large neutral amino acids (LNAAs) is another approach to diet therapy.
- **Sapropterin dihydrochloride (Kuvan),** a synthetic form of BH4, which acts as a cofactor in patients with residual PAH activity, is approved by the FDA to reduce phenylalanine levels in PKU.

Preliminary trials with recombinant phenylalanine ammonia lyase have been encouraging and demonstrated reduced blood levels of phenylalanine during treatment

54. A 40-year-old woman presents with progressive palmoplantar pigmentation X-ray spine shows calcification of IV disk. On adding benedicts reagent to urine, it gives greenish brown precipitate and blue-black supernatant fluid. What is the diagnosis? (AIIMS Nov 2008)

a. Phenylketonuria
b. Alkaptonuria
c. Tyrosinemia type 2
d. Argininosuccinic aciduria

Ans. b. Alkaptonuria

(Ref: Nelson: Defects in metabolism of Amino acids 20/e page 642)

Alkaptonuria

- Autosomal Recessive Disorder is due to a deficiency of Homogentisic Acid Oxidase
- First inborn error detected.
- Belongs to Garrod's Tetrad *Alkaptonuria, Albinism, Pentosuria, Cystinuria+

Biochemical defect

Homogentisate Oxidase deficiency leads to accumulation of Homogentisic Acid (Homogentisate) which polymerises to form Alkaptone bodies.

Clinical presentation

- Normal Life till 3rd or 4th decade.
- Urine Darkens on standing is the only manifestation in children.
- In adults Ochronosis: Alkaptone Bodies in Intervertebral Disk, cartilage of nose, pinna etc.

Laboratory Diagnosis

- Alkalanization increase darkening of urine.
- Benedicts test positive in urine because homogentisic acid is reducing agent.
- Ferric Chloride test positive
- Silver Nitrate Test positive.
- No Mental Retardation

Treatment

- New Drug is Nitisinone [NTBC] which inhibit para Hydroxyl Phenyl Pyruvate hydroxylase which prevent the accumulation of homogentisic acid.
- Symptomatic Treatment

55. Dopamine hydroxylase catalyse: (Ker 2007)

a. Dopamine to Norepinephrine
b. Dopa to Dopamine
c. Norepinephrine to Epinephrine
d. Tyrosine to Dopa

Ans. a. Dopamine to Norepinephrine

(Ref: Harper 30/e p320)

Conversion of Tyrosine to Epinephrine involves 4 sequential steps

1. Ring Hydroxylation
2. Decarboxylation
3. Side chain hydroxylation
4. N-Methylation

56. Type I Tyrosinemia is caused by: (NBE pattern Question)

a. Tyrosine Transaminase
b. Fumaryl Acetoacetate Hydrolase
c. 4-Hydroxy Phenylpyruvate Hydroxylase
d. Maleylacetoacetate Isomerase

Ans. b. Fumaryl Acetoacetate Hydrolase

(Ref: Nelson: Defects in metabolism of Amino acids 20/e page 640)

Amino acidurias and enzyme defect

Classic Phenyl Ketonuria	Phenylalanine Hydroxylase
Alkaptonuria	Homogentisate Oxidase
Tyrosinemia Type I	Fumaryl Acetoacetate Hydrolase
Tyrosinemia Type II	Tyrosine Transaminase

Contd...

Contd...

Tyrosinemia Type III	Parahydroxyphenylpyruvate hydroxylase/Parahydroxyl Phenyl Pyruvate Dioxygenase
Hawkinsinuria	Para Hydroxy Phenyl Pyruvate hydroxylase/Parahydroxylphenyl pyruvate dioxygenase is mutant, so that it catalyzes only partial reaction
Segawa Syndrome	GTP Cyclohydrolase
Albinism	Tyrosinase

57. Terminal product of Phenylalanine metabolism is: (PGI May 2014)

a. Fumarate
b. Acetyl CoA
c. Oxaloacetate

Ans. a. Fumarate, **b.** Acetyl CoA

(Ref: Harper 30/e p304)

- Terminal end products of Phenyl Alanine and Tyrosine metabolism is Fumarate, acetate and Acetyl CoA

Amino acid	Terminal end products
Asparagine, Aspartate	Oxaloacetate
Glutamine, Glutamate	α Ketoglutarate
Proline	α Ketoglutarate
Arginine, Ornithine	α Ketoglutarate
Histidine	α Ketoglutarate
Glycine, Serine	CO_2, NH_3, N_5N_{10} Methylene THFA or Pyruvate
Alanine	Pyruvate
Threonine	Glycine, Acetaldehyde
Methionine	Cysteine, Succinyl CoA
Cysteine	Pyruvate, 3 Mercaptolacetate
Phenylalanine, Tyrosine	Fumarate, Acetyl CoA, Acetate
Tryptophan	Acetyl CoA
Leucine	Acetoacetate, Acetyl - CoA
Isoleucine	Acetyl CoA, Succinyl CoA
Valine	Succinyl CoA, β Aminoisobutyrate

58. Enzyme deficiency in albinism is:

a. Tyrosinase
b. Tyrosine hydroxylase
c. Phenylalanine hydroxylase
d. Homogentisate oxidase

Ans. a. Tyrosinase

(Ref: Nelson: Defects in metabolism of Amino acids 20/e page 642)

Aminoaciduria	Enzyme deficiency
Albinism	Tyrosinase
Phenyl Ketonuria	Phenylalanine hydroxylase
Alkaptonuria	Homogentisate oxidase
Homocystinuria	Cystathionine Beta Synthase
Maple syrup Urine Disease	Branched Chain Ketoacid Dehydrogenase

59. Mousy body odor is due to: (JIPMER May 2015)

a. Phenylalanine
b. Phenylacetate
c. Phenylbutazone
d. Phenylacetylglutamine

Ans. b. Phenylacetate

(Ref: Nelson: Defects in metabolism of Amino acids 20/e page 637, 638)

Clinical Manifestations of PKU

- The affected infant is normal at birth. Profound mental retardation develops gradually if the infant remains untreated. Cognitive delay may not be evident for the 1st few months.
- Vomiting, sometimes severe enough to be misdiagnosed as pyloric stenosis, may be an early symptom.
- The infants are lighter in their complexion than unaffected siblings.
- Some may have a seborrheic or eczematoid rash, which is usually mild and disappears as the child grows older.
- These children have an unpleasant odor of **phenylacetic acid**, which has been described as musty or mousey.
- Neurologic signs include seizures (≈25%), spasticity, hyperreflexia, and tremors; more than 50% have electroencephalographic abnormalities.
- Microcephaly, prominent maxillae with widely spaced teeth, enamel hypoplasia, and growth retardation are other common findings in untreated children.

60. The amino acid that can be converted into a vitamin: (Kerala 91)

a. Glycine
b. Tryptophan
c. Phenyalalanine
d. Lysine

Ans. b. Tryptophan

- Tryptophan can be converted to Niacin.
- The rate limiting enzyme in Niacin synthesis is Quinolinate Phosphoribosyl Transferase (QPRTase)

Specialized products of Tryptophan

- Serotonin (5 Hydroxy Tryptamine)
- Melatonin
- Niacin

61. Which of the following amino acids is involved in the synthesis of thyroxine? (Karnat 97)

a. Glycine
b. Methionine
c. Threonine
d. Tyrosine

Ans. d. Tyrosine

Specialized products of Tyrosine are Melanin, Thyroxine, Catecholamines (Dopamine, Epinephrine, Norepinephrine)

62. Tyrosinemics are more susceptible to develop: (AIIMS Feb 97)

a. Adenocarcinoma colon
b. Melanoma
c. Retinoblastoma
d. Hepatic carcinoma

Ans. d. Hepatic carcinoma

(Ref: Nelson: Defects in metabolism of Amino acids 20/e page 642)

Tyrosinemia Type I (Tyrosinosis, Hereditary Tyrosinemia, Hepatorenal Tyrosinemia) Clinical Manifestations of Tyrosinemia Type I

- Untreated, the affected infant appears normal at birth and typically presents between 2 and 6 mo of age
- An acute **hepatic crisis** commonly heralds the onset of the disease and is usually precipitated by an intercurrent illness that produces a catabolic state. Fever, irritability, vomiting, hemorrhage, hepatomegaly, jaundice, elevated levels of serum transaminases, and hypoglycemia are common. An odor resembling boiled cabbage may be present, due to increased methionine metabolites. Cirrhosis and **eventually hepatocellular carcinoma** occur with increasing age. Carcinoma is unusual before two year of age.
- Episodes of acute **peripheral neuropathy** resembling acute porphyria occur in ≈40% of affected children. These crises, often triggered by a minor infection, are characterized by severe pain, often in the legs, associated with hypertonic posturing of the head and trunk, vomiting, paralytic ileus, and, occasionally, self-induced injuries of the tongue or buccal mucosa.
- **Renal involvement** is manifested as a Fanconi-like syndrome with normal anion gap metabolic acidosis, hyperphosphaturia, hypophosphatemia, and vitamin D-resistant rickets. Nephromegaly and nephrocalcinosis may be present on ultrasound examination.
- Hypertrophic cardiomyopathy and hyperinsulinism are seen in some infants.

63. Metabolites of tryptophan can give rise to: (PGI June 02)

a. Diarrhea
b. Vasoconstriction
c. Flushing
d. Can predispose to albinism
e. Phenylketonuria

Ans. a, b, c. Diarhea,Vasoconstriction, Flushing

Actions of Serotonin (Metabolite of Tryptophan) are:

- Neurotransmitter in the Brain
- Mood Elevation
- GI Motility
- Temp Regulation
- Cutaneous flushing due to vasoconstriction

64. Correct combination of Urine odor in various metabolic disorder: (PGI Nov 2013)

a. Phenylketonuria: Mousy body odor
b. Tyrosinemia: Rotten cabbage
c. Hawkinsinuria: Potato smell
d. Maple syrup disease: Rotten tomato
e. Alkaptonuria: Rotten egg

Ans. a. Phenylketonuria: Mousy body odor,
b. Tyrosinemia: Rotten cabbage

Peculiar odors in different Amino acidurias

Inborn error of metabolism	Urine odor
Glutaricacidemia (type II)	Sweaty feet, acrid
Hawkinsinuria	Swimming pool
Isovaleric acidemia	Sweaty feet, acrid
3-Hydroxy-3-methylglutaric aciduria	Cat urine
Maple syrup urine disease	Maple syrup
Hypermethioninemia	Boiled cabbage
Multiple carboxylase deficiency	Tomcat urine
Oasthouse urine disease	Hops-like
Phenylketonuria	Mousey or musty
Trimethylaminuria	Rotting fish
Tyrosinemia	Boiled cabbage, rancid butter

65. Which of the following is true regarding Phenyl Ketonuria? (PGI nov 2014)

a. Dietary Phenyl Alanine restriction is used as a treatment
b. Occur due to deficiency of Phenylalanine Hydroxylase
c. Occur due to increase activity of phenylalanine hydroxylase
d. Diet should contain high phenylalanine containing food items
e. Tyrosine should be supplied in the diet

Ans. a, b, e

- Phenylketonuria is due to deficiency of Phenyl Alanine Hydroxylase
- Dietary restriction of Phenylalanine with supplementation of Tyrosine is needed as Tyrosine is a nonessential amino acid synthesized from Phenylalanine by the action of Phenylalanine Hydroxylase.

Simple Amino acids

66. Which of the following is true about glycine? (Ker 2008)

a. Glycine is an essential amino acid
b. Sulphur containing at 4th position
c. Has a guanidine group
d. Optically inactive

Ans. d. Optically Inactive *(Ref: Harper 30/e p19)*

- **G**lycine is the only optically inactive amino acid.
- Sulfur containing amino acids are Cysteine and Methionine
- Guanidinium group is present in Arginine.
- Glycine is a nonessential amino acid

67. Which of the following would not act as source of glycine by transamination? (NBE pattern Question)

a. Alanine
b. Aspartate
c. Glutamate
d. Glyoxylate

Ans. b. Aspartate *(Ref: Harper 30/e p283)*

Biosynthesis of Glycine

- Glycine Amino Transferase catalyse the synthesis of Glycine from **Glyoxylate, Glutamate and Alanine**Q
- From **Serine** by Serine Hydroxy Methyl Transferase. This is a reversible reaction.

Serine hydroxyl Methyltransferase

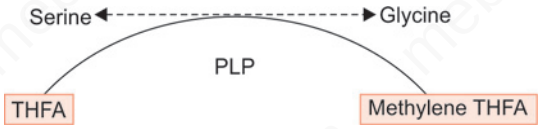


Fig. 1.44: Conversion of Serine to Glycine

- By **Glycine Synthase System** in Invertebrates
- From **Threonine** by Threonine Aldolase

68. Glycine cleavage system in liver mitochondria is associated with which enzyme? (NBE pattern Question)

a. Glycine Dehydrogenase
b. Glycine Transaminase
c. Glycine Decarboxylase
d. Glycine Dehydratase

Ans. a. Glycine Dehydrogenase

(Ref: Harper 30/e p302)

Glycine Cleavage system consists of three enzymes and an H Protein that has covalently attached Dihydrolipoyl moiety. The three enzymes are:

1. Glycine Dehydrogenase
2. Amino methyl Transferase
3. Dihydrolipomide Dehydrogenase

69. Guanidoacetic acid is formed in.....from…… (JIPMER 2000, DNB 98)

a. Kidney; Arginine + Glycine
b. Liver; Methionine + Glycine
c. Liver; Cysteine + Arginine
d. Muscle; Citrulline + Aspartate

Ans. a. Kidney; Arginine + Glycine

Steps of synthesis of Creatinine

Step I Glycine Arginine Amidotransferase

- First step in the Kidney.
- **Guanidino** group of Arginine is transferred to Glycine to form Guanidinoacetic Acid.

Step II Guanidinoacetate Methyltransferase

- Second step in the Liver
- Creatine is formed
- S Adenosyl Methionine is the methyl donor

Step III Creatine Kinase

- Third step in the Muscle
- Creatine Phosphate is formed

Step IV
- Occur spontaneously
- Creatinine is formed.

70. Conversion of glycine to serine requires: **(PGI Dec 02)**
a. Folic acid
b. Thiamine
c. Vit C
d. Fe^{2+}
e. Pyridoxal phosphate

Ans. a. Folic acid, **e.** Pyridoxal Phosphate
- Glycine is converted to Serine by Serine Hydroxy methyl Transferase
- Coenzymes required are Folic acid, Pyridoxal Phosphate

71. N Methyl Glycine is known as: **(NBE Pattern Q)**
a. Ergothionine
b. Sarcosine
c. Carnosine
d. Betaine

Ans. b. Sarcosine

Sarcosine	N Methyl Glycine
Betaine	Trimethylglycine
Choline	Trimethylethanolamine
Ethanolamine	Serine on decarboxylation
Ergothionine	Derivative of Histidine
Betamercaptoethanolamine	Cysteine on decarboxylation
Carnosine	Beta Alanyl Histidine
Anserine	Carnosine on Methylation
Homo Carnosine	GABA + Histidine
Serotonin	5 Hydroxy Tryptamine

72. What is the metabolic defect in Primary Oxaluria Type II? **(NBE Pattern Q)**
a. Glycine cleavage system
b. Alanine Glyoxalate Amino Transferase
c. D Glycerate Dehydrogenase
d. Excess Vitamin C

Ans. c. D Glycerate Dehydrogenase

Primary Hyperoxaluria Type I
- The most common form of Primary hyperoxaluria.
- It is due to a deficiency of the peroxisomal enzyme alanine-glyoxylate aminotransferase, (expressed only in the liver peroxisomes and requires pyridoxine (vitamin B_6) as its cofactor)
- Protein targeting defect.

Primary Hyperoxaluria Type II (Glyceric Aciduria)
- Due to a deficiency of D-glycerate dehydrogenase (glyoxylate reductase enzyme complex)

Secondary Hyperoxaluria
- Pyridoxine deficiency (cofactor for alanine-glyoxylate aminotransferase)
- After ingestion of ethylene glycol
- High doses of vitamin C
- After administration of the anesthetic agent methoxyflurane (which oxidizes directly to oxalic acid)
- In patients with inflammatory bowel disease or extensive resection of the bowel (*enteric hyperoxaluria*).

Nonketotic Hyperglycemia
- Due to a defect in the Glycine Cleavage System

73. All are true about glutathione except:
a. It is a tripeptide **(AIIMS Nov 2008)**
b. It converts hemoglobin to methemoglobin.
c. It conjugates xenobiotics
d. It is co-factor of various enzymes

Ans. b. Glutathione *(Ref: Harper 30/e p23)*

Functions of glutathione

Glutathione is a tripeptide
- Free radical scavenging
- Transport of Amino acid across cell membrane
- Keep iron in the ferrous sate, so prevent methmoglobin formation.
- Act as coenzyme for certain enzymes.

Phase II Xenobiotic reaction in Conjugation

Sulfur Containing Amino Acids

74. Sulfur of cysteine are not used/utilized in the body for the following process/product: **(PGI May 2015)**
a. Help in the conversion of cyanide to thiocyanate
b. Thiosulfate formation
c. Introduction of sulfur in methionine
d. Disulfide bond formation between two adjacent peptide

Ans. c. Introduction of sulfur in methionine

Methionine is an essential amino acid, so it cannot be synthesized from Cysteine.
- But Sulfur of cysteine is donated by sulfur of methionine.
- This is called transsulfuration reaction.
- PLP is the coenzyme of transrulfuration.

- The reaction is catalyzed by Cystathionine beta Synthase and Cystathionase enzyme.

75. Cysteine is abundantly found in: **(Ker 2008)**

a. Keratin
b. Chondroitin Sulfate
c. Creatine
d. Spermine

Ans. a. Keratin

- The more the disulfide bond harder the keratin is.
- Cysteine contributes to the disulfide bond.

76. N-acetyl-cysteine replenishes: **(JIPMER 2012)**

a. Glutathione
b. Glycine
c. Glutamate
d. GABA

Ans. a. Glutathione

- The active part of both Glutathione and N-Acetyl Cysteine is Sulfhydryl group of Cysteine. So N-Acetyl Cysteine replenishes Glutathione.

77. Which of the following is true about Glutathione?

a. Contain sulfhydral group **(PGI 2000)**
b. Forms met Hb from Hb
c. It does not detoxify superoxide radicals
d. Transport aminoacid across cell membrane
e. Part of enzymes

Ans. a. Contain sulfhydral group, **d.** Transport aminoacid across cell membrane, **e.** Part of enzymes

Functions of glutathione

- Free radical scavenging
- Transport of Amino acid across cell membrane
- Keep iron in the ferrous sate, so prevent methmoglobin formation.
- Act as coenzyme for certain enzymes.

78. In glutathione which amino acid is reducing agent? **(AIIMS June 1997)**

a. Glutamic acid
b. Glycine
c. Cysteine
d. Alanine

Ans. c. Cysteine *(Ref: Harper 30/e p23)*

- Glutathione is a tripeptide (Gamma Glutamic acid + Cysteine + Glycine)
- Gamma glutamyl cysteinyl Glycine
- Atypical peptide bond is present between Gamma Glutamic acid and cysteine.
- -SH (Sulfhydryl) group of cysteine is the active part of glutathione.

Acidic and Basic Amino acids

79. Nitric Oxide synthesized from?

a. Arginine **(NBE pattern Question)**
b. Citrulline
c. Alanine
d. Cysteine

Ans. a. Arginine *(Ref: Harper 30/e p661)*

Synthesis of Nitric Oxide

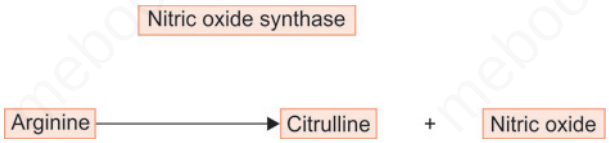


Fig. 1.45: Synthesis of nitric oxide

80. Histidine load test is used for:

a. Folate Deficiency **(NBE pattern Question)**
b. Histidine Deficiency
c. Histamine deficiency

Ans. a. Folate Deficiency *(Ref: Harper 30/e p299)*

Important Points of the histidine metabolism pathway

- Urocanate is a derivative of Histidine.
- FIGLU is Formiminoglutamic acid
- FIGLU is derived from Histidine.
- In Folic Acid deficiency FIGLU is excreted in Urine.

Histidine Load Test

- To identify Folic Acid Deficiency.
- FIGLU excreted in urine is measured following a Histidine load.

81. True about Nitric Oxide are all except: **(NBE pattern Question)**

a. Produced from arginine
b. Nitric Oxide Synthase has three isoforms
c. Otherwise called Endothelium derived Relaxing Factor
d. Acts through c AMP

Ans. d. Acts through cAMP *(Ref: Harper 30/e p661)*

- Nitric Oxide acts through cGMP
- Formed from Arginine
- iNOS, eNOS, nNOS are three isoforms of Nitric Oxide Synthase

Nitric Oxide

Uncharged molecule having an unpaired electron, so it is highly reactive, free radical.

- Very short half life (0.1 seconds)

- Formerly called Endothelium Derived Relaxing Factor.
- Gaseous molecule.
- Second messenger is cGMP.

Functions of Nitric Oxide

- Potent Vasodilator.
- Involved in Penile erection
- Neurotransmitter in brain and Peripheral Nervous System.
- Low level of NO involved in Pylorospasm in Congenital Hypertrophic Pyloric Stenosis.
- Inhibit adhesion, activation and aggregation of Platelets.

82. Creatinine is formed from: (PGI June 06)

a. Arginine
b. Lysine
c. Leucine
d. Histamine

Ans. a. Arginine *(Ref: Harper 30/e p320)*

Three amino acids from which Creatine and creatinine is synthesized are

1. Glycine
2. Arginine
3. Methionine

83. Histidine is converted to Histamine by which reaction: (NBE Pattern Q)

a. Carboxylation
b. Oxidation
c. Decarboxylation
d. Amination

Ans. c. Decarboxylation

- Amino acid is converted to ketoacid by Deamination or transamination.
- Amino acid converted to biological amines by decarboxylation.

Amino acid	Biologic amines
Histidine	Histamine
Tyrosine	Tyramine
Tryptophan	Tryptamine
Lysine	Cadaverine
Glutamic Acid[Q]	Gamma Aminobutyric Acid (GABA)
Serine	Ethanolamine
Cysteine	Betamercapto Ethanolamine

Branched Chain Amino Acid

84. Branched chain ketoacid decarboxylation is defective in: (AI 2010)

a. Maple Syrup urine disease
b. Hartnup disease
c. Alkaptonuria
d. GMI Gangliosidosis

Ans. a. Maple Syrup urine disease

(Ref: Harper 30/e p276-278)

Maple Syrup Urine Disease

Biochemical defect

- Deficiency of the enzyme Branched Chain Ketoacid Dehydrogenase.
- Defective reaction is Defective Decarboxylation.

Clinical features

- Mental Retardation
- Convulsion
- Acidosis, Coma
- Smell of Burnt Sugar [Maple Syrup]

Tests for MSUD

- Di Nitro Phenyl Hydrazine Test (DNPH Test)
- Rothera's Test
- Enzyme Analysis

Treatment

- Restrict Branched Chain Amino Acid
- Give high doses Thiamine.

85. MSUD type I A is due to mutation of:

a. E I α **(NBE pattern Question)**
b. E I β
c. E 2
d. E 3

Ans. a. E1 α

(Ref: Nelson Defects in metabolism of Amino acids 20/e page 649; Harper 30/e p311)

- **Types of MSUD[Q]**

Gene	Component	MSUD Types
E1α	Branched Chain α Keto acid decarboxylase (contains TPP)	Type I A MSUD
E1β	Branched Chain α Keto acid decarboxylase	Type I B MSUD
E2	Dihydrolipoyl Transacylase (contains Lipomide)	Type II MSUD
E3	Dihydrolipomide Dehydrogenase (Contains FAD)	Type III MSUD

86. Which is not formed from branched chain amino acid? (Latest Q)

a. Xanthurenate
b. Tiglyl CoA
c. Acetoacetyl CoA and Acetyl CoA
d. Acetyl CoA and Succinyl CoA

Ans. a. Xanthurenate *(Ref: Harper 30/e p309)*

- Xanthurenate is formed from Tryptophan if Kynureninase enzyme is defective.

87. Treatment used in Isovaleric Aciduria: (Latest Q)

a. Arginine
b. Lysine
c. Glycine
d. Methionine

Ans. c. Glycine

(Ref: Nelson's textbook of Pediatrics 20/e chapter 79.6)

Treatment of Isovaleric Acidemia

Hydration

Reversal of the catabolic state (by providing adequate calories orally or intravenously), correction of metabolic acidosis (by infusing sodium bicarbonate)

Removal of the excess isovaleric acid.

By Administering Glycine

Because isovalerylglycine has a high urinary clearance, administration of glycine (250 mg/kg/24 hr) is recommended to enhance formation of isovalerylglycine.

By administering L-Carnitine

L-carnitine (100 mg/kg/24 hr orally) also increases removal of isovaleric acid by forming isovalerylcarnitine, which is excreted in the urine.

88. Which of the following amino acid is excreted in urine in maple syrup urine disease: (AI 1999)

a. Tryptophan
b. Phenyl alanine
c. Leucine
d. Arginine

Ans. c. Leucine

Lab Diagnosis of MSUD

- Plasma shows marked elevation of leucine, isoleucine, valine, and alloisoleucine (a stereoisomer of isoleucine not normally found in blood)
- Urine contains high levels of leucine, isoleucine, and valine and their respective ketoacids

89. Diseases of branched chain amino acid includes:

a. Phenylketonuria **(PGI Nov 2013)**
b. Maple Syrup Urine Disease
c. Tay-Sachs disease
d. Isovaleric Acidemia
e. Niemann Pick disease

Ans. b. MSUD, **d.** Isovaleric Acidemia

- Phenylketonuria associated with Aromatic Amino acid
- Tay-Sach's Disease and Niemann Pick disease are Sphingolipidoses

Other Amino acids and Entry of Amino Acid to TCA Cycle

90. The nitrogen atom of aspartate formed from asparagines using enzyme asparaginase is from:

a. Ammonium **(NBE pattern Question)**
b. Glutamate
c. Glutamine
d. Alpha Ketoglutarate

Ans. c. Glutamine *(Ref: Harper 30/e p267)*

Remember
The nitrogen atom of Glutamate formed from Glutamine is from Ammonium ion.

Asparagine Synthetase

- Asparagine Synthetase is analogous to Glutamine Synthetase
- In Asparagine Synthetase, Glutamine rather than ammonium ions, provides nitrogen
- Hence cannot fix ammonia like Glutamine Synthetase
- Bacterial Asparagine Synthetase can however, also use ammonium ion

91. Oxaloacetate is formed from:

a. Proline **(NBE pattern Question)**
b. Histidine and Arginine
c. Glutamate and Glutamine
d. Aspartate and Asparagine

Ans. d. Aspartate and Asparagine

(Ref: Harper 30/e p298)

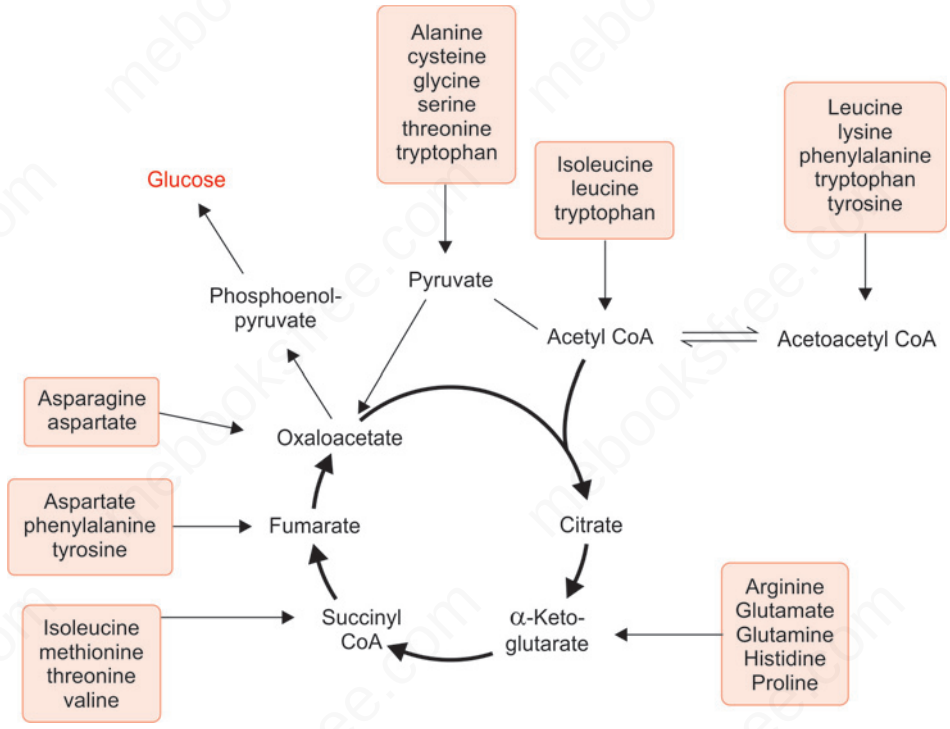


Fig. 1.46: Entry of amino acids into TCA cycle

92. Amino acid responsible for Thioredoxin reductase activation: (NBE pattern Question)

a. Serine
b. Selenocysteine
c. Cysteine
d. Alanine

Ans. b. Selenocysteine *(Ref: Harper 30/e p16)*

Selenocysteine is seen in the active site of following Enzymes and Proteins[Q]

- Thioredoxin reductase
- Glutathione Peroxidase
- Iodothyronine Deiodinase
- Selenoprotein P

93. Oxaloacetate is derived from which amino acids: (NBE pattern Question)

a. Glutamine and glutamate
b. Asparagine and aspartate
c. Histidine and arginine
d. Glutamine and Proline

Ans. b. Asparagine and Aspartate

(Ref: Harper 30/e p299)

- Asparagine and aspartate forms oxaloacetate
- Glutamine and Glutamate forms alpha Ketoglutarate.

94. Smell of sweaty feet is seen in: (NBE pattern Q)

a. MSUD
b. Phenylketonuria
c. Homocystinuria
d. Glutaric Acidemia

Ans. d. Glutaric Acidemia

(Ref: Nelson's textbook of Pediatrics 20/e page 635 table 84.3)

Peculiar odors in different Amino acidurias

Inborn error of metabolism	Urine odor
Glutaric acidemia (type II)	Sweaty feet, acrid
Hawkinsinuria	Swimming pool
Isovaleric Acidemia	Sweaty feet, Acrid
3-Hydroxy-3-methylglutaric aciduria	Cat urine
Maple syrup urine disease	Maple syrup
Hypermethioninemia	Boiled cabbage
Multiple carboxylase deficiency	Tomcat urine
Oasthouse urine disease	Hops-like
Phenylketonuria	Mousey or musty
Trimethylaminuria	Rotting fish
Tyrosinemia	Boiled cabbage, rancid butter

95. During the formation of hydroxyl proline and hydroxyl lysine, the essential factors required is/are: (PGI Dec 2003)

a. Pyridoxal phosphate
b. Ascorbic acid
c. Thiamine pyrophosphate
d. Methylcobalamine
e. Biotin

Ans. b. Ascorbic acid

- Hydroxylation of Proline and Lysine
- Enzyme: Prolyl and Lysyl Hydroxylase
- Coenzyme is Vitamin C

96. Succinyl CoA is formed by: (PGI June 1998)

a. Histidine
b. Leucine
c. Valine
d. Lysine

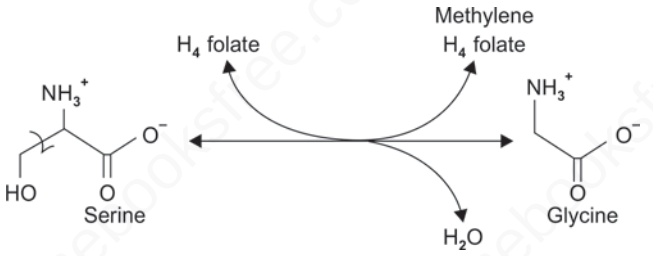
Ans. c. Valine

- Succinyl CoA is formed by Valine, Leucine, Methionine

97. In one carbon metabolism Serine loses which carbon atom? (NBE Pattern Q)

a. Alpha
b. Beta
c. Gamma
d. Delta

Ans. b. Beta *(Ref: Harper 30/e p284)*



Enzyme is Serine Hydroxymethyltransferase

Serine loses the beta Carbon atom to form Glycine and Methylene THFA.

2 Proteins

Topics Included

- Chemistry of Proteins
- Structural Organization of Proteins
- Separatory Techniques of Proteins
- Precipitation Reactions of Proteins
- Methods of Quantitation of Total Proteins.
- Protein Folding
- Glycoproteins
- Protein Sorting
- Plasma Proteins and Immunoglobulins

CHEMISTRY OF PROTEINS

Proteins are polymers of amino acid. Proteins contain carbon, hydrogen, oxygen and nitrogen as the major components. Nitrogen is characteristic of protein. On an average, the nitrogen content of ordinary proteins is 16% by weight.

Peptide Bond

All proteins are linked by peptide bond. Alpha carboxyl group of one amino acid reacts with alpha amino group of another amino acid to form a peptide bond or CO-NH bridge.

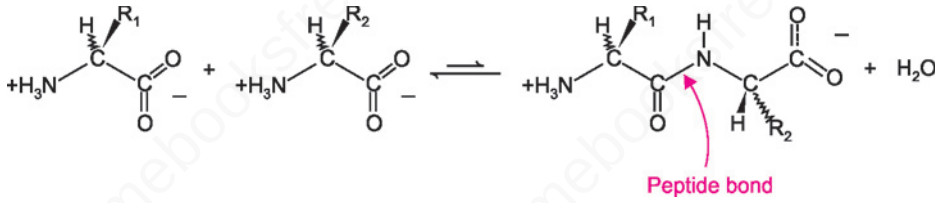


Fig. 2.1: Formation of peptide bond

Atypical Peptide Bond (Pseudopeptide Bond) (Isopeptide Bond)[PGI 2011]

An amide bond formed between an amino group and a carboxyl group at least one of which is not an alpha group. Seen in the side chains of proteins.

Bond

- *N-terminal end:* The free NH_2 group of the terminal amino acid is called as N terminal end
- *C-terminal end:* The free CO-OH end is called C-terminal end.

The amino acids are sequenced from N-terminal end to C-terminal end.

Characteristics of Peptide Bond

- Partial double bond character
- Rigid and planar
- C-N bond is trans in nature
- Uncharged but polar.

Characteristic features of an atypical peptide bond

- Occurs post-translationally
- Can be formed spontaneously or enzymatically
- Can produce stably linked protein dimers, multimers or complexes

- Makes the protein resistant as proteases cannot hydrolyse isopeptide bond.

Examples

- Glutathione
- Thyrotropin-releasing hormone
- Ubiquitin-attached to protein
- Bloodclots
- Cyclic peptide antibiotic like thyrocidin and gramicidin
- Heptapeptides like dermorphin, deltorphin.

Application of Spontaneous Isopeptide Bond Formation

- Develop a new peptide tag called Isopeptag. Used in:
  - *In vivo* protein targeting
  - Fluorescence microscopy imaging

Some Biologically Important Peptides

Peptide	Example
Tripeptide	Thyrotropin-releasing Hormone (TRH) Glutathione
Pentapeptide	Enkephalin
Octapeptide	Angiotensin-II
Nonapeptide	Oxytocin, Vasopressin [ADH], Bradykinin
Decapeptide	Angiotensin-I

CLASSIFICATION OF PROTEINS

Based on the Shape of Proteins

Fibrous Protein

- Elongated or Needle-shaped or long cylindrical or rod-like
- Minimum Solubility in water
- Regular Secondary Structure
- Axial Ratio > 10
- They are Structural Proteins
- E.g. Collagen, Elastin, Keratin.

Globular Proteins

- Spherical or Oval or Spheroidal in shape
- Easily Soluble
- Axial Ratio < 3
- They perform dynamic functions
- E.g. Albumin, Globulin, most enzymes.

Based on Nutritional Value

Nutritionally Rich (Complete Protein or First Class Proteins)

They contain all the essential amino acids in the required proportion. E.g. Casein of milk

Incomplete Protein

They lack one essential amino acid

- E.g. Pulses deficient in Methionine, Cereals deficient in Lysine.

Mnemonic: ***M****ember of* ***P****arliament takes a* ***C****asual* ***L****eave.*

Poor Protein

They lack many essential amino acids

- E.g. Zein from Corn lacks Tryptophan and Lysine.

Based on Composition

Simple Proteins

Proteins which contain only aminoacids.

- E.g. Albumin, Globulin.

Conjugated Proteins

Combination of Protein with nonprotein part called Prosthetic group.

Conjugated Protein	Constituents	Example
Glycoproteins	Proteins + Carbohydrates	Blood Group Antigens Plasma Proteins except Albumin TSH^{9}, FSH^{9}, LH^{9}
Lipoproteins	Proteins + Lipids	Chylomicrons, LDL, HDL, VLDL
Nucleoproteins	Proteins + Nucleic Acid	Histones
Chromoprotein	Proteins + Colored Prosthetic group	Hemoprotein-Hemoglobin Flavoproteins
Phosphoprotein	Contains Phosphorus	Casein of Milk, Vitellin of egg yolk
Metalloproteins	Protein + Metal ions	Tyrosinase (Contain Copper), Carbonic Anhydrase (Contains Zinc)

ORGANIZATION OF PROTEIN STRUCTURE

Proteins have different levels of structural organisation: primary, secondary, tertiary and quaternary.

Primary Structure

It is the linear sequence of amino acid held together by peptide bonds in its peptide chains. Bond involved in primary structure is Peptide Bond, a type of covalent bond.

Amino acid sequence determines the 3D structure of the protein.^{9}

Secondary Structure

- Configurational relationship between residues which are about 3–4 amino acids apart in linear sequence

- The folding of short (3- to 30-residue), contiguous segments of polypeptide into geometrically ordered units
- Bonds involved in the secondary structure are primarily **noncovalen**t bonds like:
  - Hydrogen Bond (Most important bond)
  - Hydrophobic Bond
  - Electrostatic Bond (Ionic Bond, Salt Bridges)
  - van der Waals forces.

Secondary Structures of Proteins include:
- Alpha Helix
- **Beta Pleated Sheet**
- **Loops**
- **Bends**
- **Turns**

ALPHA HELIX[Q]

- Alpha helix is the most common and stable secondary Structure
- Right-handed Spiral Structure
- Structure stabilized primarily by intrachain hydrogen bond between carbonyl oxygen of 1st and amide nitrogen of 4th amino acid
- Each turn formed by 3.6 Amino Acyl residues
- Distance of 1 turn of alpha helix (called Pitch) is 0.54 nm
  - Proline can only be stably accommodated within the first turn of an α helix
- Examples of Proteins whose major secondary structure is Alpha Helix:
  - Hemoglobin
  - Myoglobin.

BETA-PLEATED SHEET[Q]

- The second most common (hence, 'beta') recognisable regular secondary structure in proteins
- Polypeptide chain is almost fully extended
- They have a zigzag or pleated pattern
- In contrast to intrachain hydrogen bond in alpha helix, here it is interchain hydrogen bond between Carbonyl Oxygen and Amide nitrogen of two adjacent chains
- Adjacent strands in a sheet can run in the same direction (parallel) or opposite direction (antiparallel).
- ***Example of proteins whose major secondary structure is:***
  - Parallel Beta Sheet-Flavodoxin
  - Antiparallel Beta sheet-Silk Fibroin
  - Both Parallel and Antiparallel Beta sheet-Carbonic Anhydrase.

TURNS AND BENDS

Short segments of amino acid that join two units of secondary structures

Example: Beta turn.

Beta Turn

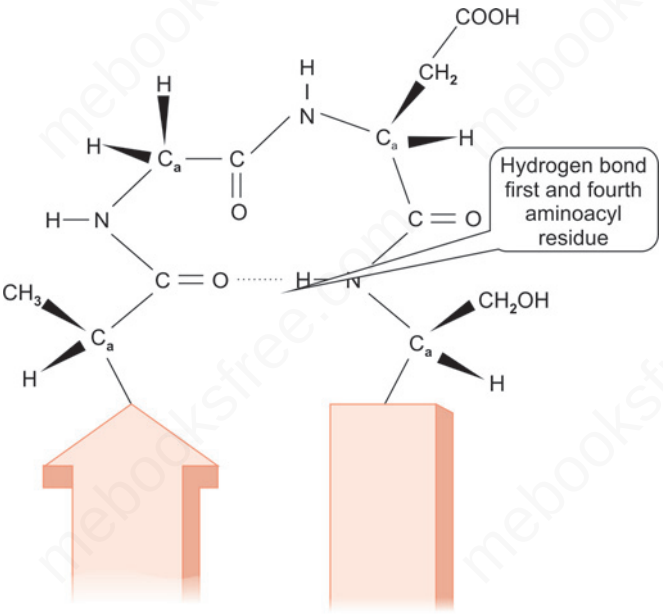


Fig. 2.2: Structure of beta turn

- Involves four aminoacyl residues
- First aminoacyl residue is hydrogen bonded to the fourth resulting in a tight 180° turn
- **Proline and Glycine** are often present in β turn.

Loops

Unlike bends and turns, loops are long segments of amino acid that join two secondary structures.

Amino acids have different propensity for forming alpha helix, Beta sheets and Beta turns

The frequency of occurrence of certain amino acid residues determines the secondary structure formed

Alpha helix
- Residues like Alanine, Glutamate, Methionine and Leucine tend to be present in the alpha helix
- Most abundant amino acid is Methionine (relative frequency is 1.47) followed by Glutamate (1.44)
- Amino acid least present in alpha helix is Proline.

Contd...

Contd...

Amino acids that do not favour alpha helix are[Q]:

- Amino acids with branches at β-carbon atom **valine, threonine and isoleucine** disrupt the stability
- Other amino acids that disrupt the stability are **Serine, Aspartate and Asparagine**
- **Proline** also disrupts the stability of alpha helix
- **Glycine** does not favor alpha helix formation.

Betasheet

- Valine and isoleucine tend to be present in beta strands
- Most abundant amino acid in beta sheet is Valine
- Amino acid least present in beta sheet is Proline

Turns

- Most abundant amino acid in turns is Proline (1.91) followed by Glycine (1.64).

SUPER SECONDARY STRUCTURE (MOTIFS)

Secondary structural elements join to form Super Secondary Structures. Examples are:

- Beta-alpha-beta motif
- Greek key motif
- Beta meander motif
- Beta barrel.

DNA-binding Motifs are examples of Super Secondary Structure. They are:

- Helix-Turn-Helix Motif
- Leucine zipper motif
- Zinc finger motif.

Points to Ponder—DNA-binding Motifs

- The first motif described is the Helix-Turn-Helix
- The second DNA binding motif is the Zinc finger
- DNA-binding Motif which requires Zinc for its activity Zinc finger
- DNA-binding Motif with leucine residues at every seventh position is Leucine Zipper.

TERTIARY STRUCTURE

The entire three-dimensional conformation of a polypeptide is referred to as tertiary structure.

Domain

A domain is a section of protein structure sufficient to perform a particular chemical or physical task, such as binding of a substrate.

Rossmann Fold

- It is a domain seen in the family of oxidoreductases
- They share a common N terminal NAD(P)+ binding region called Rossmann fold.

Oxidoreductase with Rossmann Fold[Q NBEPattern]

- Lactate dehydrogenase
- Alcohol dehydrogenase
- Glyceraldehyde-3-phosphate dehydrogenase
- Malate dehydrogenase
- Quinone oxidoreductase
- 6-phosphogluconate dehydrogenase
- D-glycerate dehydrogenase
- Formate dehydrogenase
- 3α, 20β-hydroxysteroid dehydrogenase.

QUATERNARY STRUCTURE

If more than one polypeptide aggregate to form one functional protein, the spatial relationship between the polypeptide subunits is referred to as Quaternary structure.

Bonds involved in Tertiary and Quaternary Structures are primarily noncovalent bonds.

- Hydrophobic Interaction
- Hydrogen Bond
- Electrostatic Bond
- van der Waals Forces.

Insulin has two polypeptide chains but it does not have Quaternary Structure.

In Quaternary structure, the bond involved is primarily noncovalent bond. In Insulin, two polypeptide chains are connected by disulfide bond which is a Covalent Bond. Hence even if Insulin has 2 polypeptide chains, it does not have quaternary structure.

STRUCTURE OF INSULIN

- The first Hormone to be extracted in pure form
- This was done by Banting and Best
- Banting along with the director of the institute John Macleod received Nobel Prize for the work
- The first protein in which complete sequencing was done
- Mr Frederick Sanger was the man behind this work
- He used Sanger's Reagent for this
- He won Nobel Prize for his Work
- The first protein to be produced by Recombinant DNA Technology.

Primary Structure of Insulin

- It consists of two polypeptide chains
- ***Number of Amino Acids is 51***
- A chain with 21 Amino Acids
- B chain-30 Amino Acids.

Disulfide bonds in insulin

Two interchain Disulphide Bonds:

- 7th Amino Acid in A chain to 7th Amino Acid in B chain
- 20th Amino Acid in A chain to 19th Amino Acid in B chain

One Intrachain Disulfide Bond

- 6th Amino Acid in A chain to 11th Amino Acid in A chain itself.

Species variation in insulin

- Restricted to 8, 9, 10 in A chain and C terminal Amino Acid of B chain
- Porcine and Human Insulin vary only in the terminal Amino Acid of B chain.

DENATURATION OF PROTEINS

Nonspecific alteration in secondary, tertiary and quaternary structures of protein molecule when treated with a denaturing agent.

Denaturing Agents are:

- Mild heating
- Treating with Urea
- Salicylates
- X-ray
- UV rays
- High pressure
- Vigorous shaking.

Two types of denaturation

1. *Reversible Denaturation:* Denatured proteins are sometimes renatured when physical agent is removed.
2. *Irreversible Denaturation:* Denatured proteins are not renatured when physical agent is removed.

   E.g. Albumin heated is irreversibly denatured called Heat Coagulation.

Characteristic features of denaturation are[Q]:

- Loss of Biological Activity
- Primary Structure (i.e. the peptide bond) is not altered
- Loss of Secondary and Tertiary Structures
- Loss of Folding
- They assume a Random Coil Structure.

Concept

- Everything is lost in denaturation except the primary structure (i.e. the peptide bond). Remember the peptide bond is a covalent bond, which is the strongest bond.

STUDY OF PROTEIN STRUCTURE

Study of Primary Structure/Sequencing of Proteins

Methods of protein sequencing

- End group analysis
- Mass spectrometry
- Molecular biology techniques.

End Group Analysis

Identification of N-terminal and C-terminal Amino Acid in a polypeptide chain is called end-group analysis.

Identification of N terminal amino acid

- Sanger's Technique using Sanger's reagent (1, Fluoro 2, 4 Dinitro Benzene, FDNB)[Q]
- Edman's Degradation Technique using Edman's reagent (Phenyl Isothiocyanate).

Identification of C Terminal amino acid

- Using Carboxypeptidase A and B.

Sanger's technique

- This was the first technique to determine the sequence of protein
- Sanger's Reagent is 1, Fluoro 2, 4 Dinitrobenzene[QNBE Pattern]
- Sanger's Reagent derivatizes the amino terminal residues
- The first Protein to be sequenced by the method is Insulin by Fredrick Sanger. He got Nobel prize in 1958
- Only dipeptides or tripeptides can be sequenced.

Edman's technique

- By using Edman's Reagent (Phenyl Isothiocyanate)
- Phenyl Isothiocyanate derivatizes the amino terminal of Polypeptide
- Edman's Technique can sequence many residues (5–30 residues) of a single polypeptide sample unlike Sanger's Technique.

Steps of sequencing the proteins

- To determine complete sequence of a large polypeptide, it should be first cleaved to smaller peptides. Hydrolysis of large polypeptide by using:
  - *Trypsin:* Cleave carboxyl side basic amino acids like Lysine and Arginine
  - *Chymotrypsin:* Cleave carboxyl group of amino acids, aromatic amino acids and other bulky nonpolar amino acids like Phe, Trp, Tyr, Leu, Met
  - *Cyanogen Bromide* attacks carboxyl side of methionine residue.

- Cleaved Polypeptides are purified by Reversed phase HPLC
- Short peptides are sequenced by Edman's sequencing in automated sequenator.

Mass Spectrometry

- Today mass spectrometer has emerged as the method of choice of Protein identification
- The principle used to identify protein based on mass (precisely saying on mass/charge ratio)
- The molecular mass of each amino acid is unique, the sequence of the peptide can be reconstructed from the masses of its fragments.

The exceptions are mol mass of:

- Leucine and isoleucine
- Glutamine and lysine.

Analyte has to be converted to vapor phase by using various techniques.

Methods for dispersion of analyte into vapor phase.

- Heating in a vacuum: But proteins and oligonucleotides are destroyed by heat
- Electrospray Ionization
- Matrix-Assisted Laser Desorption and Ionization (MALDI)Q
- Fast Atom Bombardment (FAB).

Types of Mass Spectrometers

Mass spectrometers come in various configurations:

- *Quadrupole mass spectrometers:* In a simple, single quadrupole mass spectrometer a sample is placed under vacuum and allowed to vaporize in the presence of a proton donor to impart a positive charge. An electrical field then propels the cations toward a curved flight tube where they encounter a magnetic field, which deflects them at a right angle to their original direction of flight. The current powering the electromagnet is gradually increased until the path of each ion is bent sufficiently to strike a detector mounted at the end of the flight tube. **For ions of identical net charge, the force required to bend their path to the same extent is proportionate to their mass.**
  - Quadrupole mass spectrometers generally are used to determine the masses of molecules of **4000 Da or less.**

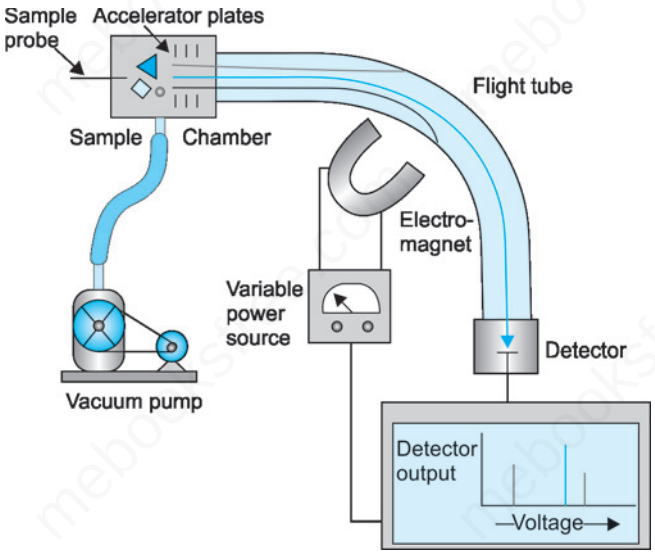


Fig. 2.3: Mass spectrometer

- *Time of flight (TOF) mass spectrometer:* Following vaporization of the sample in the presence of a proton donor, an electric field is briefly applied to accelerate the ions toward the detector at the end of the flight tube. For molecules of identical charge, the velocity to which they are accelerated—and hence the time required to reach the detector—is inversely proportional to their mass. The flight tube for a time-of-flight (TOF) mass spectrometer is linear. No magnetic field to deflect the path of the sample is required.
  - Time-of-flight mass spectrometers are used to determine the large masses of complete proteins (> 4KDa)
- *Tandem mass spectrometry:* Two mass spectrometers are linked in series. For this reason, such tandem instruments are often referred to as MS–MS.

Advantages of Mass Spectrometers

- *Method of choice:* Protein determination
- Superior sensitivity, speed and versatility
- Detect posttranslational modification unlike Edman's reaction and DNA-derived Protein sequence
- Can be used for other biomolecules like oligonucleotide, carbohydrates as mass and charge are common properties of all this.

Molecular Biology Revolutionized the Determination of Primary Structure

- The basic principle of Edman's chemistry is employed to sequence a small portion of protein

- Using the data from genetic code, remaining sequence is determined.

Study of secondary structure

- Circular Dichroism
- Optical Rotatory Dispersion Chromatography.

Study of tertiary structures

- X-ray Crystallography
- NMR Spectroscopy
- UV Light Spectroscopy (Most rapid method)
- Fluorescence Spectroscopy
- Molecular Modeling.

SEPARATORY TECHNIQUES OF PROTEINS

Different separatory techniques of proteins are:

- Salt fractionation
- Ultracentrifugation
- Electrophoresis
- Chromatography.

Salt Fractionation (Salting Out)

Principle

The solubility of proteins is generally lowered at high salt concentrations, an effect called 'salting out'. The addition of a salt in the right amount can selectively precipitate some proteins, while others remain in solution. Ammonium sulfate ($(NH_4)_2SO_4$) is often used for this purpose because of its high solubility in water.

Ultracentrifugation

Principle

Method to separate the protein based on the mass, density, to an extent to the shape also.

Electrophoresis

Migration of charged particle in an electric field is called electrophoresis.

Concept of Electrophoresis

- Sample is applied usually at the cathode end and the analyte moves towards the anode
- So negatively charged particles move faster
- If a mixture of amino acids is separated, then the negatively charged amino acid moves faster.

Classified based on the supporting media used to separate the analyte of interest.

Type of electrophoresis	Supporting media used	Property used to separate the proteins
Agarose gel electrophoresis	Agarose gel	Based on charge
Cellulose acetate electrophoresis	Cellulose acetate membrane	
Polyacrylamide gel electrophoresis (PAGE)	Polymer of acrylamide	Based on charge and molecular weight (size)
Sodium dodecyl sulfate (SDS)—PAGE	Sodium dodecyl sulfate and polyacrylamide SDS imparts equal negative charge so that it masks the inherent charge of the protein. Now proteins separate based on size only	Based on molecular weight (size)
Capillary electrophoresis	Separation done in a capillary tube	Based on charge
Isoelectric focusing	Supporting media with pH gradient	Based on isoelectric pH

Two-dimensional Electrophoresis

In one direction SDS-PAGE and in the other direction, Isoelectric focusing. So, separation is based on both molecular weight (size) and isoelectric pH.

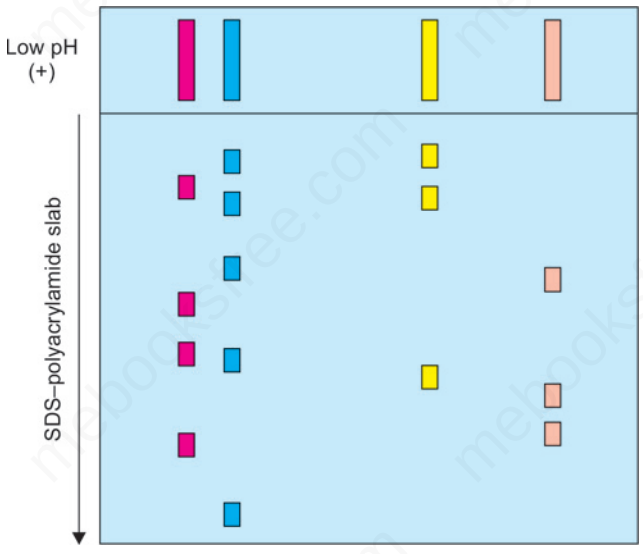


Fig. 2.4: Isoelectric focusing

High Pressure Liquid Chromatography (HPLC)

Versatile technique among the Column methods of Chromatography.

Concept of HPLC

A modern refinement in chromatographic methods is **HPLC,** or **high-performance liquid chromatography.** HPLC makes use of **high-pressure** pumps that **speed the movement of the protein** molecules down the column, as well as higher-quality chromatographic materials that can withstand the crushing force of the pressurized flow. **By reducing the transit time** on the column, HPLC can **limit diffusional spreading** of protein bands and thus **greatly improve resolution.**

Advantages

- Separation can be based on different property based on the column (affinity chromatography, ion exchange columns, size exclusion, etc. used
- Better Resolution and Less Transit Time
- Reproducible Results
- Hence High Performance (So also called High Performance Liquid Chromatography)
- For separating Hemoglobin fraction HPLC[Q] is used.

Densitometry

Method to quantitate the separated protein.

Chromatography

Method of separation of proteins based on the differential distribution of analyte between stationary and mobile phase.

Concept of Various Chromatographic Techniques

Thin Layer/Paper Chromatography

Stationary phase is water held on support media and mobile phase is a mixture of nonpolar solvents. The sample is applied on the support media. As the nonpolar solvent ascend on the stationary phase, the nonpolar component of the analyte move along the mobile phase, but polar component will remain in the stationary phase.

Size Exclusion Chromatography

The column matrix is a cross-linked polymer with pores of selected size. Larger proteins migrate faster than smaller ones, because they are too large to enter the pores in the beads and hence, take a more direct route through the column. The smaller proteins enter the pores and are slowed by their more labyrinthine path through the column. So, separation is based on molecular weight or size of the particle.

Affinity Chromatography

The beads in the column have a covalently attached chemical group. A protein with affinity for this particular chemical group will bind to the beads in the column, and its migration will be retarded as a result. So this is based on the biological activity.

Ion Exchange Chromatography

This can be Anion exchange or Cation exchange Chromatography. In cation-exchange chromatography, the solid matrix has negatively charged groups. In the mobile phase, proteins with a net positive charge migrate through the matrix more slowly than those with a net negative charge, because the migration of the former is retarded more by interaction with the stationary phase. In anion exchange chromatography it is vice versa. So, separation is based on the charge of the analyte.

Various Chromatographic Techniques

Classified based on the stationary medium (Column) used for separation

Chromatography	Stationary phase used	Property used for separation
• **Paper chromatography**	• Water held on a solid support of filter paper (or Cellulose)	• Based on the polarity • Least Polar moves faster
• **Thin layer chromatography**	• Silica gel (Kieselguhr) spread on a glass plate or a plastic sheet or aluminium sheet.	• Based on Polarity • Least Polar moves faster
• **Ion exchange chromatography**	• Column of Ion exchange resins • Anion exchange or Cation exchange resins	• Based on Charge-Charge Interaction
• **Size exclusion chromatography** **Other names:** • Molecular sieve chromatography • **Gel filtration chromatography** • **Gel permeation chromatography**	• Column of porous beads	• Based on molecular weight (size) • Particles emerge in the descending order of Stokes Radius[Q]
• **Affinity chromatography**[Q]	• Column of resins bound to specific ligands used	• Based on specific ligand-binding behavior or biological activity
• **Hydrophobic interaction chromatography**		• Based on hydrophobic interaction.
• **Absorption chromatography**		• Based on absorption property

Compare the Different PAGEs

PAGE

- Protein is separated based on molecular mass or molecular weight/ size and charge.

SDS-PAGE

- SDS imparts equally negative charge so that it masks the inherent charge of the Protein
- Now Proteins separate based on molecular weight (size) only.

SDS-PAGE in conjunction with 2 mercaptoethanol or dithiothreitol

- Oxidatively cleave disulfide bond
- So, separate the components of multimeric proteins.

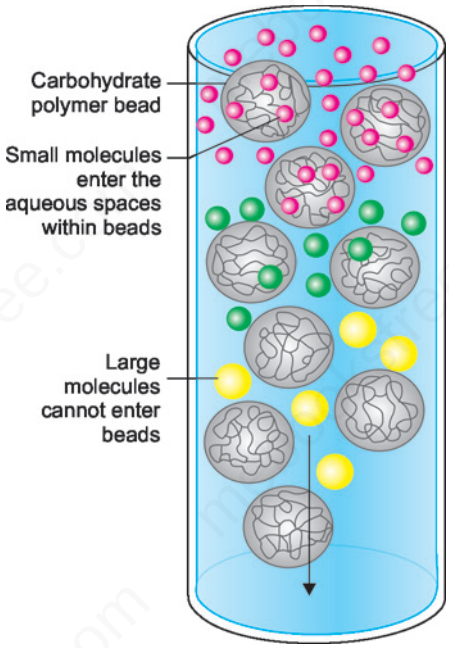


Fig. 2.5: Size exclusion chromatography

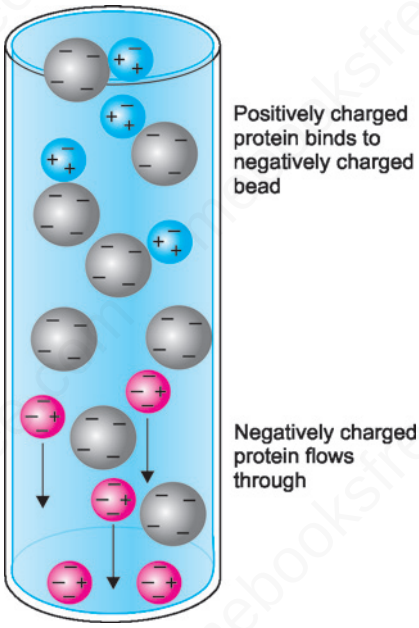


Fig. 2.6: Ion exchange chromatography

Remember

- Most specific Chromatographic technique is Affinity Chromatography
- Most rapid method of separation of Proteins is Capillary Electrophoresis
- Method to quantitate the separated protein is Densitometry
- Hydrophobic amino acid moves fastest along the stationary phase of Thin Layer Chromatography or Paper Chromatography.

Contd...

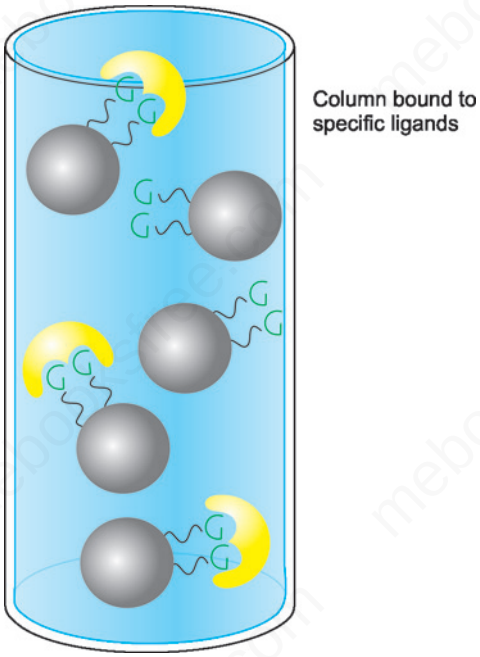


Fig. 2.7: Affinity chromatography

Contd...

- The amino acid which moves fastest in a Thin layer Chromatography/ Paper Chromatography is Isoleucine
- SDS PAGE used in conjunction 2 Mercaptoethanol or dithiothreitol break component polypeptides of multimeric proteins
- In electrophoresis, negatively charged amino acids and proteins move faster
- In paper and thin layer chromatography, nonpolar amino acids move faster.

PRECIPITATION REACTIONS OF PROTEINS

Polar groups of proteins tend to attract water molecules around them to produce a shell of hydration. This makes the protein soluble in water. Any factor that neutralizes the charge of protein or remove the shell of hydration will cause precipitation of proteins.

Methods to precipitate protein by neutralizing the charge are

- Precipitation by heavy metallic salt
- Precipitation by acids.

Methods to precipitate protein by removing the shell of hydration

- Precipitation by neutral salts
- Precipitation by organic solvents

- **Precipitation by heavy metallic salt**
  - Heavy metals like Hg, Zn, Pb, etc. provide positively charged ions and neutralise the negative charge of the protein
  - Thus precipitate the protein.

  - The reagents used are Mercuric nitrate, Zinc sulphate, Lead acetate, Ferric cholride.
- **Precipitation by acids**
  - Acids bring the pH of the medium to isoelectric pH, precipitability is maximum at isoelectric pH. This is because at isoelectric pH, proteins carry no net charge; hence no shell of hydration
  - The reagents used are Phsophotungstic acid, Sulphosalicylic acid, Phosphomolybdic acid, Trichloroacetic acid.
- **Precipitation by neutral salts**
  - Concentrated salt solution removes the shell of hydration
  - Reagents used are ammonium sulfate. This is called salting out.
- **Precipitation by organic solvents**
  - Organic solvents reduce the dielectric constant of water and decreases the water available for protein, hence it is precipitated
  - The reagents used are ether, alcohol, acetone, etc.

METHODS OF QUANTITATION OF TOTAL PROTEINS

Kjeldahl's Procedure

Principle: *The nitrogen present in the protein is reduced to ammonia, which is absorbed in acid medium and estimated.*

Advantages and Disadvantages

- Most specific and accurate method but takes many days to get the result.

Biuret Method

Principle: Cupric ions chelate with peptide bonds of protein in alkaline medium to produce violet color. The intensity of color is used to quantitate the protein.

Advantages and Disadvantages

- Simple method and most widely used
- But sensitivity is less.

Lowry's Method

Principle: Based on reduction of Folin-Ciocalteau phenol reagent (Phosphomolybdic acid and Phosphotungstic acid) Tyrosine or Tryptophan residues of protein.

Advantages and Disadvantages

- Very sensitive method
- Microgram quantity of protein is estimated
- But depends on Tyrosine and Tryptophan residues in protein.

Spectrophotometric Estimation

Principle: Based on absorbtion of uv light at 280 nm by aromatic amino acid.

Advantages and Disadvantages

- Most accurate, simple and highly sensitive
- But instrument is costly.

Radial Immunodiffusion [Mancini'sTechnique] QNBE Pattern

Principle: On agar gel with specific antibody, wells containing antigen are made. Antigen moves radially and a white ring of precipitate is obtained. The diameter of precipitation ring will be proportional to the log of antigen concentration.

Advantages

- Simple, sensitive technique
- Specific as it is based on antigen antibody interaction.

Bradford Assay

Principle: Dye-binding assay of protein using Coomassie Brilliant Blue. The change in color on dye binding is assayed colorimetrically or spectrophotometrically.

Light-scattering Techniques

Principle: Based on measurement of scattered light by antigen antibody complexes.

Two methods:

1. *Nephelometry:* Scattered light at 60° is measured.
2. *Turbidimetry:* Scattered light at 180° is measured.

Advantages and Disadvantages

- Rapid method
- Suitable for automated methods
- Instruments and reagents are costly.

RIA and ELISA

Advantages

Nanogram or Picogram quantities of proteins can be measured.

Remember

Bromo Cresol Green (BCG) Method is a method to estimate total albumin AND NOT total protein. Densitometry quantitates the separated protein in an electrophoretogram.

IMMUNOCHEMICAL METHODS

Immunochemical methods forms the basis of a diverse range of sensitive and specific clinical assay known as immunoassay. In immunoassay an antibody is used as a reagent to detect the analyte (antigen) of interest.

Advantages of Immunoassays

Exquisite specificity and high affinity of antibodies for antigen make immunoassays highly specific and sensitive.

Various immunochemical techniques:

- Passive Gel Diffusion
- Immunoelectrophoresis
- Western Blot.

Passive Gel Diffusion

- *Single Immunodiffusion:* Concentration gradient established for single reactant. Quantitative technique based on this is Radial Immuno Diffusion (RID)
- *Double Diffusion:* Concentration gradient is established for both antigen and antibody. This technique is widely used and is known as **Ouchterlony Technique**.[QNBE Pattern]

Immuno Electrophoresis (IEP)

Technique used to separate and identify various protein species contained in spinal fluid, serum etc.

- **Crossed Immuno Electrophoresis (CRIE):** Electrophoresis used in second dimension to drive antigen in to gel containing antibodies specific for the antigen of interest[Q]
- **Counter Immuno electrophoresis (CIE):** Two parallel lines of wells are punched into the agar. One row is filled with antigen solution and the opposing row is filled with antibody solution. Voltage is applied across the gel to cause the antigen and antibody to move towards each other at a faster rate. A precipitin line is formed where they meet.

FIBROUS PROTEINS

Collagen[QQQ]

- The major structural protein found in extracellular matrix (Connective tissue)
- Most abundant protein in the body
- Present in all the tissues of the body
- Highest concentration in the Skin (74%), followed by Cornea (64%).

Types of Collagen[Q]

- Major collagen present in bone-Type I (90%)
- Major collagen present in dermis, ligaments and tendons-Type I (80%)
- Major collagen present in cartilage-Type II (40–50%)
- Major collagen present in hypertrophic cartilage-Type X
- Major collagen present in aorta-Type I and Type III (20–40% each)
- Major collagen present in basement membrane-Type IV
- Major collagen present in skin hemidesmoses-Type XVII
- Major collagen present in rhabdomyosarcoma cells-Type XIX
- Most abundant collagen-Type I.

Structure of Collagen

Characteristic features

Glycine-X-Y repeats

Every third amino acid residue in collagen is a glycine residue.

Alpha chain

- Polyproline helix of three residues per turn twisted in **left-handed direction**
- Each polypeptide chain contains 1000 amino acids.

Triple helical structure

Three of these alpha chains are then wound into a **right-handed superhelix.**

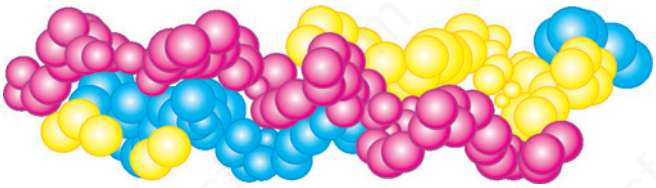


Fig. 2.8: Triple helix–collagen

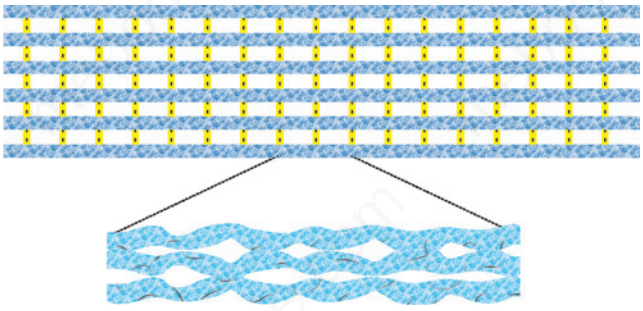


Fig. 2.9: Covalent cross links and quarter staggered arrangement of collagen

'Quarter Staggered' arrangement[Q]

- ***Lateral association of the triple helical units***
- Each is displaced longitudinally from its neighbor by slightly less than one-quarter of its length
- Responsible for tensile strength of collagen fibers.

Synthesis of Collagen

Can be divided into:

- Intracellular events:
  - Inside the fibroblast
  - Procollagen is formed
- Extracellular events
  - Tropocollagen is formed.

Intracellular Events[Q]

- Cleavage of signal peptide
- Hydroxylation of prolyl residues and some lysyl residues
- Glycosylation of some hydroxylysyl residues
- Formation of intrachain and interchain S–S bonds in extension peptides
- Formation of triplehelix.

Extracellular Events[Q]

- Cleavage of amino and carboxyl terminal propeptides
- Assembly of collagen fibers in quarter-staggered alignment
- Oxidative deamination of amino groups of lysyl and hydroxylysyl residues to aldehydes.
- Formation of intra and interchain cross-links via Schiff bases and aldol condensation products.

Unique Events in Collagen Formation

- Hydroxylation
  - Posttranslational modification occurring intracellularly
  - Enzyme: Prolyl and Lysyl Hydroxylase
  - Coenzyme: Vitamin C (Ascorbic Acid) and alpha Ketoglutarate
  - Essential for the three chains of the monomer to fold into a triple helix at body temperature.
- Glycosylation
  - Intracellular event
  - Hydroxylysine residues are glycosylated with galactose or glucose
  - **By type III O-glycosidic linkage.**
- Oxidative Deamination
  - Extracellular event
  - Enzyme: Lysyl Oxidase
  - Cofactor: Copper
  - Reaction: Oxidative deamination of Lysyl and Hydroxy Lysyl residues to form Aldehydes.
- Covalent Crosslinks
  - Covalent Cross links by Aldol Condensation of modified Lysyl and Hydroxy Lysyl residues by oxidative deamination.
  - Provide tensile strength to Collagen.

Diseases Associated with Collagen[Q]

Type of collagen	Gene or enzyme	Disease
Type I	COL1A1 and COL 1A2	Osteogenesis Imperfecta Osteoporosis Ehlers-Danlos Syndrome (Type VII EDS)
Type II	COL2A1	Chondrodysplasias Osteoarthritis
Type III	COL3A1	Ehlers-Danlos syndrome (Type IV EDS)
Type IV	COL4A3–COL4A6	Alport syndrome (including both autosomal and X-linked forms)
Type V and Type I	COL 5A1, COL5A2 COL1A1	Classical EDS
Type III	COL3A1 Tenascin XB (TNXB)	Hypermobile EDS (Type III EDS)
Type VII	COL7A1	Epidermolysisbullosa, dystrophic
Type X	COL10A1	Schmid metaphysial chondrodysplasia
Lysyl hydroxylase	Lysyl hydroxylase	Ehlers-Danlos syndrome (Type VI EDS) (Kyphoscoliotic EDS) Scurvy
ADAM Metallopeptidase with Thrombospondin Type I motif (ADAM TS2) also called Procollagen N-proteinase	Procollagen N-proteinase	Ehlers-Danlos syndrome (Type VII autosomal recessive) Dermatosparaxis type
Lysyloxidase	Lysyloxidase	Menke's disease

CLINICAL CORRELATION–FIBROUS PROTEINS

Ehlers Danlos Syndrome (EDS) or Cutis Hyper Elastica Different Forms of EDS (Imp for AIPGMEE/DNB)

Classical EDS[Q]

- Type I EDS (Severe) and Type II EDS (Mild)
- They have Classical Clinical features.

Classical manifestation:

- Hyperelasticity of Skin (hence called Rubber Person Syndrome)
- Hypermobile joints
- Affect Type V Collagen and Type I Collagen.

Hypermobile EDS (Type III EDS)

- Joint hypermobility is more prominent than skin changes.

Vascular EDS (Type IV EDS)

- *Most serious EDS*
- Type III Collagen is affected
- Skin changes are more prominent than joint changes.
- Predisposed to sudden death from rupture of large blood vessels or other hollow organs.

Type V EDS

- Inherited as an X-linked trait

EDS with Tenascin X Defect seen in

- Recently included in hypermobile EDS (type III).

Tenascin X

- Protein coded by TNXB gene
- Minor component of connective tissue that appears to regulate the assembly of collagen fibers.

EDS due to enzyme defect

- Arthrochalasic EDS (type VII EDS) or Dermatosparaxis type
  - Mutations in procollagen N-proteinase (ADAM TS2)
  - Very fragile and sagging skin
- Ocular-scoliotic type of EDS (type VI EDS) or Kyphoscoliotic
  - Mutations in the PLOD 1 gene, which encodes procollagen-lysine 5-dioxygenase (lysylhydroxylase)
    - Scoliosis
    - Ocular fragility
    - Keratoconus (Cone-shaped deformity of the cornea)
- Progeroid form of EDS
  - Mutations in B4GALT7, the gene for β -1, 4-galactosyltransferase 7.

The Villefranche Classification of Ehlers Danlos Syndrome

Subtype	Defect in
Hypermobility	Type III collagen, tenascin X
Classical	Types I and V collagen
Vascular	Type III collagen
Kyphoscoliosis	Lysylhydroxylase
Arthrochalasis	Type I collagen
Dermatosparaxis	ADAM metallopeptidase with thrombospondin type 1 motif (ADAMTS2)

Points to ponder–EDS

- Most Common Collagen affected is Type III Collagen.
- Most common inheritance is Autosomal dominant.
- Most serious is Type IV EDS (Vascular) affecting Type III Collagen.

Alport's Syndrome (AS)[Q] (Hereditary Nephritis)

- X-linked disorder
- Type IV Collagen is affected
- Clinical features
  - Hematuria
  - Sensorineural deafness
  - Conical deformation of the anterior surface of the lens (lenticonus).

The pathognomonic of classic Alports Syndrome lenticonus together with hematuria

Electron microscopy reveals abnormality in the structure of basement membrane and lamina densa.

Achondroplasia

- Best known cause of Chondrodysplasia
- Most common cause of short limb dwarfism
- Caused by mutation in a gene that codes for the receptor for fibroblast Growth factor-3 (FGFR-3).

Strickler Syndrome

- An example of Chondrodysplasia.
- Characterized by degeneration of joint cartilage and vitreous body of eyes.

ELASTIN

- A connective tissue protein that is responsible for properties of extensibility and elastic recoil in tissues.
- Present in lung, large arterial blood vessels, and some elastic ligaments.

Oxidative Deamination and Desmosine[QAIIMS] Cross Links in Elastin

Enzyme-lysyl oxidase

- Lysyl residues of tropoelastin are oxidatively deaminated to aldehydes
- Condensation of three of these lysine-derived aldehydes with an unmodified lysine to forma tetrafunctional cross-link unique to elastin.

Hydroxylation in Elastin

Proline hydroxylated to hydroxyproline by Prolyl hydroxylase.

Differences Between Collagen and Elastin

Collagen	Elastin
Different types of Collagen present	Only one type exist.
Triple helix Structure	No triple helix instead random coil conformations
(Gly-X-Y) n repeating structure	No (Gly-X-Y) n repeating structure
Presence of hydroxylysine	No hydroxylysine
Glycosylation present	No Glycosylation
Intramolecular aldol cross-links	Intramolecular desmosine cross-links
Presence of extension peptides during biosynthesis	No extension peptides present during biosynthesis

Clinical Correlation-Elastin

Mutations in the elastin gene (ELN) leads to:

- Supravalvular aortic stenosis **(William Beuren Syndrome)**
- Cutis laxa.

FIBRILLIN

- Glycoprotein
- Structural component of microfibrils
- Secreted into the extracellular matrix by fibroblasts
- Incorporated into the insoluble **microfibrils**
- Provide a **scaffold** for deposition of elastin
- Fibrillin-1 is the major fibrillin present
- Fibrillin-2 is important in deposition of microfibrils early in the development.

Clinical Correlation-Fibrillin

Marfan Syndrome

By mutations in the gene (on chromosome 15) for fibrillin-1.

Triad of Marfan's Syndrome

- Skeletal changes
- *Ectopia lentis*
- Aortic aneurysms.

Biochemical basis of characteristic clinical features in Marfan Syndrome

Explained by location of Fibrillin 1

- Zonular fibers of the **lens,** hence ectopialentis
- **Periosteum,** hence arachnodactyly
- Elastin fibers in the **aorta,** hence aortic aneurysms.

Ghent Criteria

An international Criteria to classify the Marfan Syndrome.

Remember

Fibrillin-1 gene mutation is also recently found to be associated with

- Acromicric dysplasia
- Geleophysic dysplasia.

Congenital Contractural Arachnodactyly

- Mutation in Fibrillin 2 located in Chromosome 5
- Fibrillin-2 may be important in deposition of microfibrils early in development
- Presence of contractures.

Loeys-Dietz Syndrome (LDS)

- Mutation in Transforming Growth factor Beta Receptors (TGFBR1 and TGFBR2)
- Characterized by aortic aneurysms, cleft palate, and hypertelorism
- Similar to Marfan's syndrome except in the absence of ocular changes in LDS.

KERATIN

- Alpha helix coiled coil structure, i.e. two alpha helix are wind around one another to form a super helix
- They belong to the family of Intermediate Filament. (IF)
- As it is an alpha helix coiled coil, Keratin is rich in hydrophobic amino acids like Ala, Leu, Met, Val, Phe.
- Cross links are formed by disulphide bond
- Cysteine is involved in the disulphide bond
- The more the Disulphide bond, the harder the Keratin
- Protein present in the hair nails and outer layer of skin.

Clinical Correlation-Keratin

Epidermolysis Bullosa Simplex

Mutations in the genes for the major keratins of basal epithelial cells (keratins 5 and 14).

LAMININ

- It is a major protein component of Renal Glomerular and Other Basal Laminas
- Elongated cruciform shape.

The primary components of the basal lamina are three proteins:

1 Laminin.
2 Entactin.
3 Type IV collagen.

Glycosamino Glycans present in the Basal Lamina are:

1 Heparin.
2 Heparan sulphate.

Entactin (also known as 'nidogen')

- Is a glycoprotein containing an RGD (Arginine, Glycine and Aspartic Acid) sequence
- It binds to laminin
- Is a major cell attachment factor.

PROTEIN FOLDING

Proteins are conformationally dynamic molecule that can fold into functionally competent conformation. Auxillary Proteins assist Protein Folding, they are called Chaperones.

Properties of Chaperone Proteins[Q]

Present in a wide range of species from bacteria to humans

- Many are so-called **Heat Shock Proteins (Hsp)**
- Are inducible by conditions that cause unfolding of newly synthesized proteins (e.g. elevated temperature and various chemicals)
- They bind to predominantly hydrophobic regions of unfolded proteins and prevent their aggregation
- They act in part as a quality control or editing mechanism for detecting misfolded or otherwise defective proteins
- Most chaperones show associated ATPase activity.

Molecular Chaperones are (Very imp topic)

- HSP 70
- HSP 90
- HSP 40 [Cochaperone]
- BiP [Immunoglobulin heavy chain binding protein]
- Glucose Regulated Protein [GRP-94]
- Calreticulin
- Calnexin.

Enzymes assist folding are:

- Protein Disulphide Isomerase[Q]
- Peptidyl Prolyl Isomerase.[Q]

Chaperonins

- The second major class of chaperones
- HSP 60 family of chaperones, sometimes called **chaperonins**
- They form complex **barrel-like structures** in which an unfolded protein is retained, giving it time and suitable conditions in which to fold properly
- For example, mtGroEL chaperonin.

ROLE OF ENDOPLASMIC RETICULUM (ER) IN PROTEIN FOLDING (IMP TOPIC FOR AIIMS AND PGI)

The ER functions as the quality control compartment of the cell.

The newly synthesized proteins after entering ER attempt to fold with the assistance of chaperones and folding enzymes in the lumen of ER.

ER Stress Sensors Initiate Unfolded Protein Responses (UPR)

- Mechanism to sense the levels of misfolded proteins and initiate intracellular signaling mechanisms to compensate for the stress conditions and restore ER homeostasis is Unfolded Protein Response (UPR)
- UPR increases the ER folding capacity and prevents a build up of unproductive and potentially toxic protein products.

ERAD (Endoplasmic Reticulum Associated Degradation)

- Misfolded or incompletely folded proteins interact with chaperones, which retain them in the ER and prevent them from being exported to their final destinations
- The misfolded proteins are usually disposed of by **Endoplasmic Reticulum Associated Degradation (ERAD)**. Explained later.

PROTEIN DEGRADATION

- Intracellular proteases hydrolyze internal peptide bonds
- The resulting peptides are then degraded to amino acids
  - By endopeptidases that cleave internal peptide bonds
  - By amino peptidases and carboxypeptidases that remove amino acids sequentially from the amino- and carboxyl-termini, respectively.
- ***PEST sequences, regions rich in proline (P), glutamate (E), serine (S), and threonine (T), target some proteins for rapid degradation.***

Two Types of Proteins Degradation

1. ATP Independent.
2. ATP Dependent.

ATP Independent Degradation

Proteins that undergo ATP independent degradation are:

- Extracellular proteins
- Membrane-association proteins
- Long lived intracellular proteins.

Site: Lysosomes

By ATP independent mechanism.

For example, Blood glycoprotein.

ATP Dependent Degradation

Proteins that undergo ATP dependent degradation:

- Regulatory proteins with short half-lives
- Abnormal or misfolded proteins.

Site: In the cytosol by proteasomal complex:

- ATP dependent mechanism
- Requires Ubiquitin.

This is also termed as ERAD [Endoplasmic Reticulum Associated Degradation of Proteins]

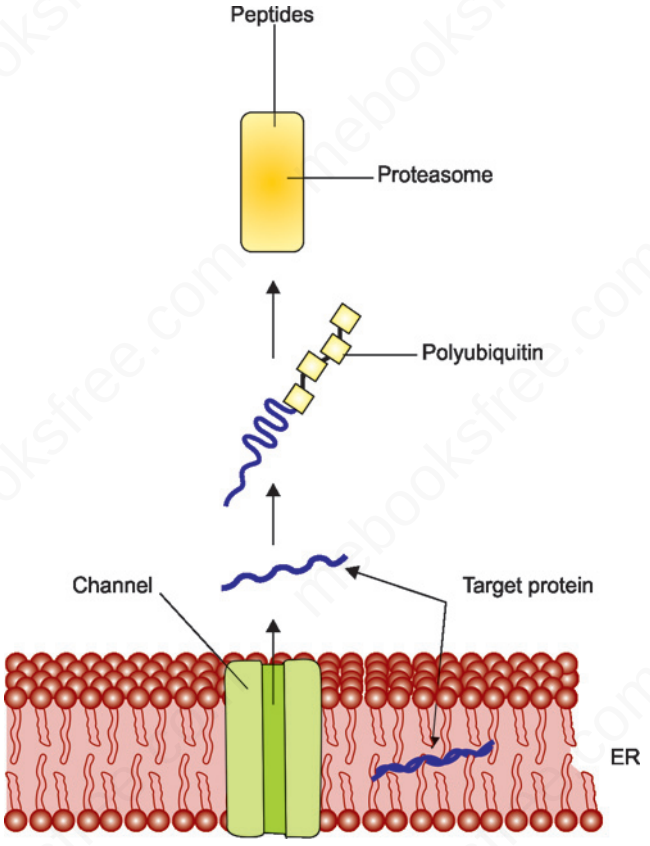


Fig. 2.10: Endoplasmic reticulum associated protein degradation

Steps of ERAD

- A target protein which is misfolded undergoes retrograde transport through the ER (Retrotranslocation or dislocation) membrane into the cytosol
- It is subjected to polyubiquitination
- Then enters a proteasome, inside which it is degraded to small peptides that exit and may have several fates.

Transmembrane proteins that helps in the retrotranslocation of misfolded membrane and luminal protein in ER to cytosol are:

- Sec61 (Translocon)
- Degradation in ER protein1 (derlin 1)
- ERAD E3 ligases
- Hrd1
- Doa10

Ubiquitin

- Key molecule in protein degradation
- Small protein with 76 Amino Acids
- Highly conserved protein
- Attachment of Ubiquitin to Protein to be degraded is called **Kiss of Death**
- Ubiquitin bind to the ε amino group of Lysine of the target protein hence it is a **Pseudopeptide or Isopeptide Bond or non α peptide bond**
- Minimum of **four ubiquitin** molecules must be attached to commit target molecule to degradation.

N end rule of ubiquitin binding

Ubiquitin bind to proteins with PEST [Proline, Glutamic Acid, Serine and Threonine] sequence in the amino terminal.

Proteasome

- Ubiquitinated proteins are degraded in Proteasome
- Located in the Cytosol
- Large cylindrical structure composed of 50 sub-units
- This is an ATP dependent process.

Structure of Proteasome

Proteasomes are protein complexes

It is a large cylindrical structure

It is composed of:

- Four rings with a hollow **core** containing the protease active sites
- One or two **caps** or **regulatory particles** that recognize the polyubiquinated substrates.

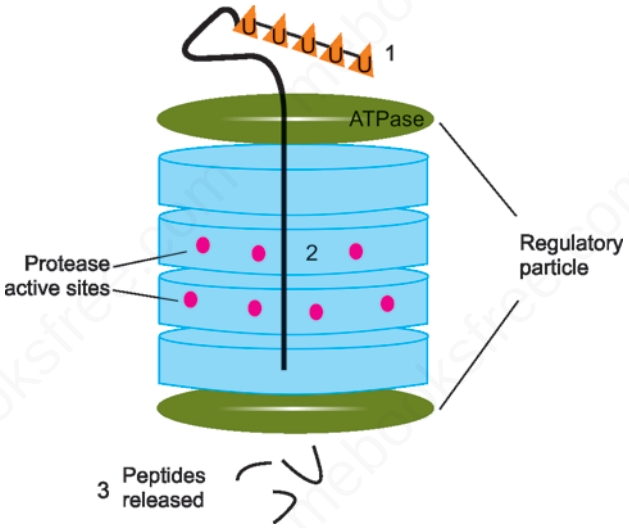


Fig. 2.11: Structure of proteasome and steps of proteasomal degradation

Steps of Proteasomal Degradation

- The regulatory particle recognizes the ubiquitinated protein which are unfolded by ATPases present in the regulatory particles or caps
- Protease active sites in the core of the proteosome attack peptide bonds and degrade the protein
- Peptides are released into the cytosol for further degradation by cytosolic peptidases.

Clinical Correlation
Proteasome Inhibitor [Bortezomib]
Used in Multiple Myeloma
For Hepatocellular Carcinoma

PROTEIN MISFOLDING DISORDERS

- Prion Diseases
- Prion Related Protein Diseases.

Human Prion Diseases

Disease	Host	Mechanism of pathogenesis
Kuru	Fore people	Infection through ritualistic cannibalism
Creutzfeldt-Jakob disease (CJD)		
Iatrogenic CJD	Humans	Infection from prion-contaminated hGH, duramater grafts, etc.
Variant CJD	Humans	Infection from bovine prions

Contd...

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Disease	Host	Mechanism of pathogenesis
Familial CJD	Humans	Germ-line mutations in PrP gene located in Chromosome 20
Sporadic CJD	Humans	Somatic mutation or spontaneous conversion of cellular isoform of the prion protein (PrPC) into disease-causing isoform of the prion protein (PrPSc)
Gerstmann-Sträussler-Scheinker (GSS) disease	Humans	Germ-line mutations in PrP gene located in Chromosome 20
Fatal Familial Insomnia (FFI)	Humans	Germ-line mutation in PRNP
Sporadic Familial Insomnia	Humans	Somatic mutation or spontaneous conversion of PrPC into PrPSc

Points to Remember
- The most common prion disorder in humans—Sporadic CJD (sCJD)
- The most common etiology of Prion Diseases—Sporadic (85%)
- The second most common etiology of Prion Diseases—Germline Mutation (10–15%)
- The Prion Diseases with Noninfectious etiology are:
  - sCJD
  - fCJD
  - Gerstmann-Sträussler-Scheinker (GSS) disease.
  - Fatal Familial Insomnia (FFI)

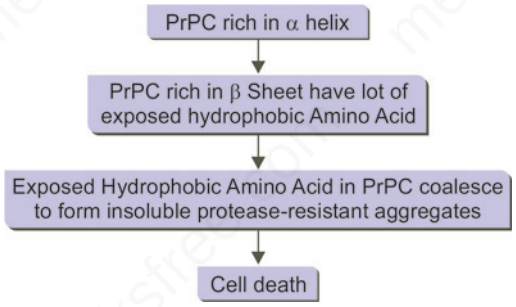
Some Terms to Remember

PRNP	PrP gene located on human chromosome 20.
PrP	Human prion-related protein
PrPC	Cellular isoform of the prion protein ***Monomeric and rich in α helix***
PrPSc	Disease causing isoform of the prion protein. • *Is rich in β sheet*

Biochemical Basis of Prion Diseases

By a Conformational Chain Reaction

One pathologic prion or prion-related protein can serve as template for the conformational transformation of many times its number of PrPc molecules.



The Prion Related Protein Diseases[Q]

Prion like changes underlie many neurodegenerative

Basic mechanism is protein rich in α helix changes to protein rich in β sheet.

Diseases like

- Alzheimers Disease
- Parkinson's Disease
- Huntington's Disease
- Fronto Temporal Dementia
- Dementia with Lewy Bodies
- Amyloidosis
- Beta thalassemia.

Prion related Protein diseases and abnormally aggregated Proteins

Disease	Abnormally aggregated protein
Alzheimer's Disease	Aβ42 Tau
Huntington's Disease	Huntingtin
Fronto Temporal Dementia [FTD]	Tau Inclusions Pick Bodies TDP-43 inclusions FUS inclusions
Dementia with Lewy Bodies [DLB]	α-synuclein inclusions (Lewy bodies)

Beta-Thalassemias

- Thalassemias are caused by genetic defects that impair the synthesis of one of the polypeptide subunits of hemoglobin
- During the burst of hemoglobin synthesis that occurs during erythrocyte development, a specific chaperone called α-Hemoglobin-Stabilizing Protein (AHSP) binds to free hemoglobin α-subunits awaiting incorporation into the hemoglobin multimer
- In the absence of this chaperone, free α-hemoglobin subunits aggregate, and the resulting precipitate has cytotoxic effects on the developing erythrocyte.

AMYLOIDOSIS

Amyloidosis is the term for diseases caused by the **extracellular deposition** of insoluble polymeric **protein fibrils** in tissues and organs.

The term amyloid was coined by the pathologist Rudolf Virchow.

Amyloid Fibrils

Physical Nature of Amyloid

X-ray crystallography and infrared spectroscopy demonstrate a characteristic **cross-β pleated sheet conformation.**

Congo red staining shows **apple-green birefringence** under polarized light.

By electron microscopy amyloid is seen to be made up largely of **continuous, nonbranching fibrils** with a diameter of approximately 7.5 to 10 nm.

Chemical Nature of Amyloid

- 95% of the amyloid material consists of fibril proteins
- 5% of the amyloid material consists of P component and other glycoproteins.

Nomenclature of Amyloid Fibrils

The accepted nomenclature is AX, where:

- A indicates amyloidosis
- X represents the protein in the fibril.

Common Amyloid Fibril and its Characteristics

AL amyloid

- Seen in primary systemic amyloidosis
- The first major Amyloid fibril Protein
- Composed of immunoglobulin light chains (LCs)
- Associated with a clonal B cell disorder and may be associated with myeloma or lymphoma.

AA amyloid

- Seen in secondary amyloidosis
- The second major class of amyloid fibril protein
- Is composed of the acute-phase reactant serum amyloid A protein
- AA Amyloid is derived by proteolysis from a larger (12,000 daltons) precursor in the **serum called SAA (serum amyloid–associated) protein that is synthesized** in the liver
- Associated with chronic inflammatory or infectious diseases.

Aβ amyloid

- Seen in localized amyloidosis
- Aβ is the most common form of Localised Amyloidosis
- Aβ protein is derived by proteolysis from a much larger transmembrane glycoprotein, called amyloid precursor protein or Amyloid β Protein
- Aβ is deposited in the brain in Alzheimer's disease.

ATTR amyloid

- Seen in familial Amyloidoses and Systemic Senile Amyloidosis Composed of Transthyretin.

Transthyretin (TTR)

- Normal serum protein that binds and transports thyroxine and retinol.

In Familial Amyloidosis (Familial amyloidotic neuropathies)

- A mutant form of TTR (and its fragments) is deposited.

In systemic senile amyloidosis

- Normal TTR is deposited in the heart of aged individuals.

β2M amyloid

- Seen in Hemodialysis associated Systemic Amyloidosis
- Composed of β2–2-microglobulin, a component of MHC class I molecules occurs in individuals with End-Stage Renal Disease (ESRD) of long duration
- In patients on long-term hemodialysis.

Protein misfolding results in Amyloidosis

The proteins that form amyloid fall into two general categories:

- Increased production of **normal proteins** that have an inherent tendency to fold improperly, associate and form fibrils. Examples:
  - SAA is synthesized by the liver cells under the influence of cytokines such as IL-6 and IL-1 that are increased during long standing inflammation leads to **AA Amyloid**
  - **Immunoglobulin light chain** synthesized by the plasma cells increased in Monoclonal B lymphocyte proliferation results in **AL Amyloid**
- Mutant proteins that are prone to misfolding are produced and subsequent aggregation. (NB:-No increased production). Example:
  - Mutant TTR aggregation in Familial amyloidosis.

Systemic Amyloidosis

Amyloidosis	Chemically related precursor protein	Clinical syndrome	Organ involvement
Systemic Generalized Amyloidosis			
AL	Immunoglobulin Light Chain chiefly λ type	Primary or myeloma associated or Immunocyte dyscrasias with amyloidosis	Any organs
AH	Immunoglobulin heavy chain	Primary or myeloma associated or Immunocyte dyscrasias with amyloidosis	**Any organs**
AA	Serum amyloid A protein	Secondary or Reactive Systemic	Renal or Any
Aβ2M	β2-Microglobulin	Hemodialysis-associated	Synovial membrane, bone

Contd...

Contd...

Amyloidosis	Chemically related precursor protein	Clinical syndrome	Organ involvement
Systemic Senile Amyloidosis			
ATTR	Transthyretin	Senile systemic (wild type)	Cardiac
Hereditary Amyloidosis (Familial Amyloidosis) (Heredofamilial Amyloidosis)			
ATTR	Transthyretin	Familial (mutant) Familial amyloidotic neuropathies	Cardiac, peripheral and autonomic **nerves**
AA	Serum Amyloid A Protein	Familial Mediterranean fever	Renal or Any
A ApoA1	Apolipoprotein A1	Familial	Hepatic, renal
A ApoAII	Apolipoprotein A1	Familial	Renal
A Gel	Gelsolin	Familial	
Corneas, cranial nerves, renal			
A Fib	Fibrinogen Aα	Familial	Renal
A Lys	Lysozyme	Familial	Renal
ALECT2	Leukocyte chemotactic factor 2	?	Renal

Localized Amyloidosis

Amyloidosis	Chemically related precursor protein	Clinical syndrome	Organ involvement
Aβ	Amyloid protein	Alzheimer's disease;	Down syndrome
ACys	Cystatin C	Cerebral amyloid angiopathy	CNS, vascular
APrP	Prion protein	Spongiform encephalopathies	CNS
AIAPP	Islet amyloid polypeptide (amylin)	Diabetes-associated	Islets of Langerhans of Pancreas
ACal	Calcitonin	Medullary carcinoma of the thyroid	Thyroid
AANF	Atrial natriuretic factor	Age-related Isolated Atrial Amyloidosis	Cardiac atria
APro	Prolactin	Endocrinopathy	Pituitary

Heredofamilial Amyloidosis

- **Familial mediterranean fever**
  - The most common and best studied
  - An autosomal recessive condition
  - The gene for familial Mediterranean fever encodes a protein called *pyrin* (for its relation to fever), which is one of a complex of proteins

that regulate inflammatory reactions via the production of proinflammatory cytokines
  - This disorder is encountered largely in individuals of Armenian, Sephardic Jewish, and Arabic origins
  - The amyloid fibril proteins are made up of AA proteins
- **Familial Amyloidotic Polyneuropathies**
  - An autosomal dominant familial disorder
  - Characterized by deposition of amyloid made up of mutant TTRs
  - Predominantly in peripheral and autonomic nerves.

IMMUNOGLOBULINS

Immunoglobulins, are synthesized mainly in plasma cells (specialized cells of B cell lineage) in response to exposure to a variety of antigens.

Structure of Immunoglobulin

It consists of 2 heavy chains and 2 light chains

Light chain and Heavy chain are divided into Constant Region towards the Carboxyl end and Variable Region towards the Amino terminal end.

- In the Light chain VL and CL
- In the Heavy chain CL and C_H1, C_H2, C_H3.

Hinge Region

The region between the C_H1 and C_H2 domains.

The hinge region confers flexibility and allows both Fab arms to move independently, thus helping them to bind to antigenic sites.

Heavy Chain Types in Immunoglobulins

Based on the heavy chain types Immunoglobulins are divided into five classes.

- IgG γ-Chain
- IgA α-Chain
- IgM μ-Chain
- IgD δ-Chain
- IgE ε-Chain.

Light Chain Types in Immunoglobulins

Two types:

- ***κ and λ light chain.***
- In a given immunoglobulin either 2κ or 2λ and never a mixture of κ and λ
- Most abundant light chain in humans is κ.

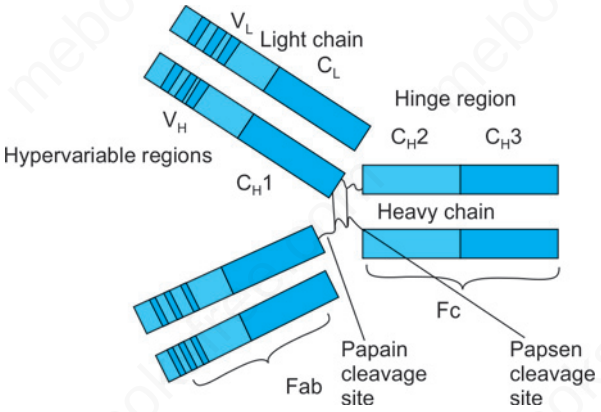


Fig. 2.12: Structure of immunoglobulin

Variable Regions of Immunoglobulin

Consist of the VL and VH domains

They are quite heterogeneous.

Variable regions are comprised of

- **Hypervariable Regions**
  - Hypervariable regions comprise the antigen-binding site (Fab)
  - Hypervariable regions are also termed complementarity-determining regions (CDRs).
- **Relatively Invariable Regions**
  - The surrounding polypeptide regions between the hypervariable regions are termed as frame work regions.

Proteolytic Cleavage of Immunoglobulin

- **Papain Digestion**
  - Site of Cleavage-Beyond the Disulphide Bond in the Hinge Region
  - Products of Cleavage-2 separate Fab fragments and 1 Fc fragment.
- **Pepsin Digestion**
  - Site of Cleavage-Before the disulphide bond in the hinge region
  - Products of Cleavage-Bivalent Fab fragments, $F(ab)_2$ and digested Fc fragments.

Fc and Fab fractions of Immunoglobulin

- **Fab (Fraction Antibody)**
  - Fragment that bind with Antigen
  - Located in the variable region of Heavy and Light Chain.
- **Fc (Fraction Crystallizable)**
  - Remaining part of the Immunoglobulin molecule
  - Concerned with activation of Complement Cascade.

CLASSES OF IMMUNOGLOBULIN AND ITS CHARACTERISTICS

Immunoglobulin G (IgG)

- Monomer
- Most Versatile as IgG can perform all the functions of Immunoglobulin
- Major Immunoglobulin in the Serum (75–80%)
- Subclasses are IgG1, IgG2, IgG3 and IgG4
- Most abundant IgG Subclass is IgG1 (50%)
- Main antibody in secondary immune response
- IgG is the only class of Ig that crosses the placenta. Transfer is mediated by a receptor on placental cells
- Only IgG Subclass that do not cross the placenta is IgG2
- Complement activation is present in the order of IgG3 > IgG1 > IgG2 > IgG4
- Good For opsonization as Fc receptor present on phagocytic cells
  - IgG1 and IgG3 good for opsonization
  - IgG2 and IgG4 less affinity for Fc receptors.

Immunoglobulin A (IgA)

Two types:

1. Serum IgA—Monomer.
2. Secretory IgA —Dimer joined by J Chain.

- The main effector of the mucosal immune system
- The most abundant immunoglobulin of body secretions such as saliva, tears, colostrum and gastrointestinal secretions
- Normally IgA does not fix complement, unless aggregated [? IgA Alternate pathway].

Secretory Component (Piece) of Secretory Immunoglobulin is made in mucosal epithelial cells is added to the IgA as it passes into the secretions.

Function of secretory piece

- Protects it from degradation in the secretions
- Ensures, the appropriate tissue localization of SIgA by anchoring the antibody to mucus lining the epithelial surface.

Immunoglobulin M (IgM)

- Pentamer joined by J chain
- Largest Immunoglobulin
- IgM is the first Immunoglobulin to be made by the fetus
- Immunoglobulin involved in primary Immune response
- Most effective activator of Classical Complement pathway.

Immunoglobulin D (IgD)

- Found in low levels in serum
- Role in serum uncertain
- Primarily found on B cell surfaces where it functions as a receptor for antigen
- Does not bind complement.

Immunoglobulin E (IgE)

- Cytophilic Antibody
- Least common Immunoglobulin in the serum
- Involved in allergic reactions as a consequence of its binding to basophils and mast cells
- IgE also plays a role in parasitic helminth diseases
- Does not fix complement.

Quick Revision Immunoglobulins

- Most Common Immunoglobulin present in the serum-IgG
- Least Common Immunoglobulin in the serum-IgE
- Largest Immunoglobulin-IgM
- Immunoglobulin which is a pentamer-IgM
- Immunoglobulin which is a dimer-Secretory IgA
- **Immunoglobulin which fixes the complement-IgG and IgM**
- ? IgA by Alternate Pathway
- Immunoglobulin which is present in Secretions-Secretory IgA
- Shape of an Immunoglobulin monomer-Y Shape
- Immunoglobulin which crosses placenta-IgG
- IgG which do not crosses the placenta-IgG2
- Immunoglobulin involved in Primary Immune response-IgM
- Immunoglobulin involved in Secondary Immune response-IgG
- Immunoglobulin with J Chain-IgM and IgA
- Immunoglobulin with secretory piece-IgA.

PLASMA PROTEINS

By using the method of Salting out Plasma Proteins can be divided into three fragments:

1. Fibrinogen.
2. Albumin.
3. Globulins.

By using the Electrophoresis technique Serum Proteins can be divided into five bands. They are (from anode to cathode)

1. Albumin.
2. α1 Globulins.
3. α2 Globulins.
4. β Globulins.
5. γ Globulins.

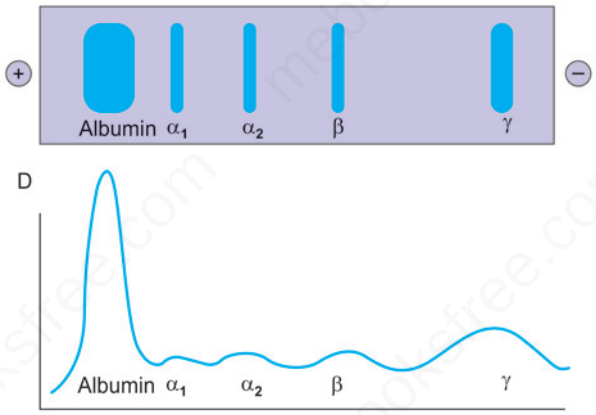


Fig. 2.13: Electrophoretic patterns of plasma proteins

Abnormal Electrophoretic Patterns

- *Chronic infection:* The gamma globulins are increased as smooth and wide based band
- *Multiple myeloma:* Sharp band in the gamma globulins called M band due to monoclonal origin of Immunoglobulins
- *Nephrotic syndrome:* Alpha 2 fraction is most prominent as all other small proteins lost through urine
- *Cirrhosis:* Albumin band is thin.

Instead of Serum Electrophoresis if Plasma Electrophoresis done. What is the difference in electrophoresis pattern?
To answer this question first know the difference between Serum and Plasma
Blood collected without adding anticoagulants is Serum and with anticoagulants is Plasma
Serum = Plasma-Clotting factors
In Plasma Electrophoresis: Fibrinogen with other clotting factors form a prominent band in the Gamma region Clinical Significance: Confused with M Band in Multiple Myeloma.

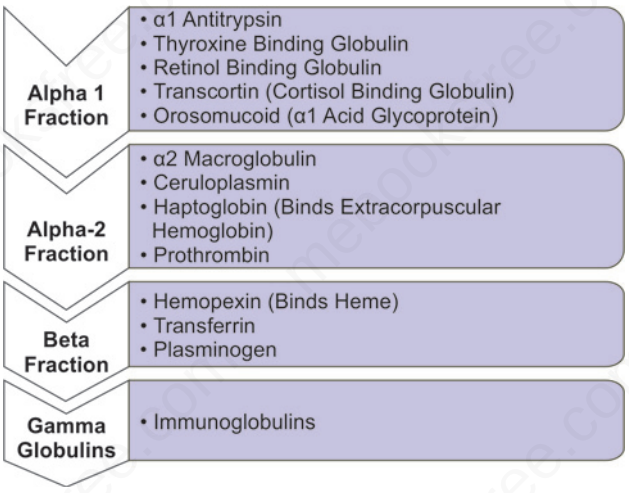


Fig. 2.14: Plasma proteins present in each fractions of electrophoretogram

Prealbumin or Transthyretin
- Slightly faster mobility than Albumin fraction in electrophoresis
- Major role in transport of Thyroxine and retinol
- Associated with Familial and Senile Amyloidosis.

Functions of Plasma Proteins

Function of the plasma protein	Plasma protein
Maintain the colloid osmotic Pressure of the Plasma.	Albumin
Nutritional Function	Albumin
Buffering Action in the Plasma	Albumin
Anti proteases	Antichymotrypsin α1-Antitrypsin (α1-antiproteinase) α2-Macroglobulin Antithrombin
Blood clotting	Various coagulation factors, fibrinogen
Hormones	Erythropoietin
Immune defence	Immunoglobulins, complement proteins, α2-microglobulin
Involvement in inflammatory responses	Acute phase response proteins (e.g. C-reactive protein, α1-acid glycoprotein [orosomucoid])
Oncofetal	α1-Fetoprotein (AFP)

Plasma Proteins in Transport Function[Q]

Transport protein	Compound it binds
Albumin	• Bilirubin, • Free fatty acids • Ions [Ca^{2+}] • Metals [e.g. Cu^{2+}, Zn^{2+}], • Metheme • Steroids • Hormones
Ceruloplasmin	• Cu^{2+}
Corticosteroid-binding globulin (transcortin)	• Cortisol
Haptoglobin	• Extracorpuscular hemoglobin
Lipoproteins	• Plasma Lipids
Hemopexin	• Heme
Retinol-binding protein	• Retinol
Sex-hormone-binding globulin	• Testosterone • Estradiol
Thyroid-binding globulin	• T_4 • T_3
Transferrin	• Iron
Transthyretin (formerly prealbumin)	• T_4 and forms a complex with retinol-binding protein

Quick Review of Plasma Proteins

- Most abundant Plasma Protein-Albumin
- Least abundant Plasma Protein–Alpha-1 Globulin
- Plasma Protein with fastest electrophoretic mobility-Prealbumin (Transthyretin) followed by albumin. Plasma Protein with least electrophoretic mobility-Gamma Globulin
- Most Plasma Proteins are synthesized in the liver except Immunoglobulins by Plasma cells of B Lymphocyte lineage
- Most Plasma Proteins are Glycoproteins except Albumin, which is a Simple protein. Many Plasma Proteins exhibit Polymorphism. They are:
  - α1-antitrypsin
  - Haptoglobin
  - Transferrin
  - Ceruloplasmin
  - Immunoglobulins.

ACUTE PHASE REACTANTS

Group of plasma proteins whose concentration increases or decreases in response to inflammatory and neoplastic conditions.

Positive Acute Phase Reactants

Plasma proteins whose concentration increases in response to inflammatory and neoplastic conditions.

- C reactive Protein
- Ceruloplasmin
- Haptoglobulin
- Fibrinogen
- α1 Acid Glycoprotein
- α1 antiprotease.

Negative Acute Phase Reactants

Plasma proteins whose concentration decreases in response to inflammatory and neoplastic conditions.

- Albumin
- Transthyretin
- RBP
- Transferrin.

CLINICAL CORRELATION–PLASMA PROTEINS

Plasma cell Disorders

Monoclonal Neoplasm arising from common pro-genitors in the B lymphocyte lineage. Synonyms are:

- Monoclonal Gammopathy
- Paraproteinemias
- Plasma cell Dyscrasias
- Dysproteinemias.

Biochemical Investigations in Plasma Cell Disorders

Serum protein electrophoresis and densitometry

- Sharp spike (Church Spire Spike) in the globulin region is called a ***M component*** **(M for monoclonal)**
- The minimum concentration of monoclonal antibody for M Component to be seen is 5 g/L (0.5 g/dL)
- This is a method for **quantitative** assessment of the M component
- The amount of M component in the serum is a reliable measure of the tumor burden. This makes the M component an excellent **tumor marker**
- In approximately 1% of patients with myeloma, **biclonal or triclonal** gammopathy is observed.

M Component detected in other diseases

Lymphoid Neoplasms
- Chronic lymphocytic leukemia
- Lymphomas of B or T cell origin.

Nonlymphoid Neoplasms
- Chronic myeloid leukemia
- Breast cancer
- Colon cancer.

Nonneoplastic Conditions
- Cirrhosis
- Sarcoidosis
- Parasitic diseases
- Gaucher disease
- Pyodermagangrenosum.

Autoimmune Conditions
- Rheumatoid arthritis
- Myasthenia gravis
- Cold agglutinin disease.

Skin Diseases
- Lichen myxedematosus (Papular mucinosis)
- Necrobiotic xanthogranuloma.

- **Immunoelectrophoresis**
  - This is a method for qualitative assessment of Immunoglobulins
  - The nature of the M component is variable in plasma cell disorders
  - Type of immunoglobulin is determined by immunoelectrophoresis
  - IgG myelomas (53%) are more common than IgA and IgD myelomas.
- **Urine Bence Jones Protein (BJP)**
  - They are Monoclonal Immunoglobulin light chain excreted in urine
  - Seen in 20–30% of Myeloma.

Bradshaw's Test: Test to detect urine BJP

- Urine layered over a few ml of concHCl—White ring of Precipitate.

Special heating test for urine BJP

- This test is based on special property of BJP
- BJP precipitate when heated between 45–60°C but redissolve when heated > 80°C and < 45°C
- 50% False Negative Test
- **Serum Free Light Chain Assay**
  - New Technique to quantitate Serum light chain.
- **Serum Alkaline Phosphatase (ALP)**
  - It is normal since there is no osteoblastic activity.
- **Serum β2 Microglobulin**
  - Single most powerful predictor of Survival
  - Can substitute for staging:
    - Levels < 0.004 g/L have a median survival of 43 months
    - Levels > 0.004 g/L have a median survival of 12 months.

Staging System in Multiple Myeloma

- **Durie-Salmon staging system**
  - Previously used is not widely used nowadays.
- **International Staging System [ISS]**
  - Most widely used method of assessing prognosis
  - Parameters employed in ISS are:
    - Beta 2 Microglobulin (β2M)
    - Albumin (alb).

International Staging Systems

Parameters	Stage	Median survival, months
β2M < 3.5, alb ≥ 3.5	I (28%)	62
β2M < 3.5, alb < 3.5or β2M = 3.5–5.5	II (39%)	44
β2M > 5.5	III (33%)	29

RNA- and DNA-based Prognostic Staging Systems

- Microarray profiling for RNA Based Staging System
- Comparative genomic hybridization for DNA based staging system.

Quick review points-Plasma cell disorders

- Patients secreting lambda light chains have a significantly shorter overall survival than those secreting kappa light chains.
- IgM Myeloma has the greatest tendency for Hyperviscosity.
- Among IgG myelomas, it is the IgG3 subclass that has the highest tendency to hyperviscosity and cold agglutination.
- High labeling index and high levels of lactate dehydrogenase are also associated with poor prognosis.

Diagnostic Criteria of Plasma Cell Disorders

Multiple Myeloma

- M Protein in serum and/or urine
- Bone Marrow Plasma cells
- Myeloma related organ or tissue impairment (end organ damage, including bone lesions)

Myeloma Variants

Monoclonal Gammopathy of Undetermined significance (MGUS)

- Most common plasma cell disorder
- M Protein in serum < 30g/L
- Bone marrow clonal plasma cells < 10%
- NO myeloma related organ or tissue impairment (end organ damage, including bone lesions)

Smoldering Myeloma (Asymptomatic Myeloma)

- M Protein in serum > 30 g/L
- Bone marrow clonal plasma cells > 10%
- NO myeloma related organ or tissue impairment (end organ damage, including bone lesions)

Nonsecretory Myeloma

- No M Protein in serum or Urine
- Bone marrow clonal plasma cells > 10%
- Myeloma related organ or tissue impairment (end organ damage, including bone lesions)

Solitary Plasmacytoma of Bone

- No M Protein in serum or urine
- Single area of bone destruction due to clonal plasma cells
- Bone marrow not consistent with Multiple Myeloma
- NO myeloma related organ or tissue impairment (end organ damage, including bone lesions)

OTHER PLASMA CELL DISORDERS

Waldenstroms Macroglobulinemia[Q]

- A malignancy of lymphoplasmacytoid cells that secreted IgM
- It originates from a postgerminal center B cell has the characteristics of an IgM-bearing memory B cell
- Lymphadenopathy and Hepatosplenomegaly
- Hyperviscosity syndrome present
- Raynaud's phenomenon present
- No bone lesion, renal involvement. Or hypercalcemia
- Sia test Positive
- Coombs' test Positive.

POEMS Syndrome[Q]

- **P**olyneuropathy
- **O**rganomegaly [Hepatomegaly Lymphadenopathy, Splenomegaly]
- **E**ndocrinopathy
- **M**ultiple myeloma
- **S**kin changes.

Heavy Chain Disease

Gamma Heavy Chain Disease (Franklin's Disease)

- Lymphadenopathy in Waldeyer's Ring
- Palatal Oedema characteristic.

Alpha Heavy Chain Disease (Seligmann's Disease)

- Most common heavy chain disease
- Closely related to Mediterranean Lymphoma
- Infiltration of lamina propria of small intestine with Truncated Alpha Chain
- No sharp peak on electrophoresis
- Mu Heavy Chain Disease
- The secretion of isolated mu heavy chains.

Immunoproliferative Small Intestinal Disease (IPSID)

- An infectious pathogen–associated human lymphoma that has association with Campylobacter jejuni
- Affect Proximal Small Intestine
- Excessive Plasma cell differentiation-producing truncated alpha heavy chain lacking light chain and 1st constant domain.

α1 Antitrypsin (α1 Antiproteinase) Deficiency

- **α1-Antiproteinase** was formerly called α1–antitrypsin
- The major component (> 90%) of the α1 fraction of human plasma. It is synthesized by hepatocytes and macrophages
- The principal serine protease inhibitor (serpin, or Pi) of human plasma
- It inhibits trypsin, elastase, and certain other proteases, hence accurately called α1Antiproteinase
- The major genotype is **MM**, and its phenotypic product is PiM.

Two disorders associated with α1 Antitrypsin (α1 Antiproteinase) Deficiency:

1. Emphysema
2. Cirrhosis of Liver.

Emphysema

- Five percent of **emphysema is associated with** α1 Antitrypsin deficiency
- This occurs mainly in subjects with the ZZ genotype, who synthesize PiZ, and also in PiSZ heterozygotes
- Both of whom secrete considerably less protein than PiMM individuals
- Polymorphonuclear white blood cells increase in the lung (e.g. during pneumonia)
- The affected individual lacks a countercheck to proteolytic damage of the lung by proteases such as elastase.

Active Elastase + α1AT → Inactive Elastase α1 AT Complex → No Proteolysis of Lung

Active Elastase + Decreased α1 AT→Active Elastase → Proteolysis of Lung → Emphysema

Treatment

- Intravenous administration of α1-antitrypsin (augmentation therapy)
- Gene therapy.

Smokers have more chance for developing Emphysema

- **Methionine** residue at 358th position of α1-antitrypsin is involved in its binding to **proteases.**
- **Smoking** oxidizes this methionine to methionine sulfoxide and thus in activates it.
- As a result, affected molecules of α1-antitrypsin no longer neutralize proteases.
- This is particularly devastating in patients (e.g. PiZZ phenotype) who already have low levels of α1-antitrypsin.
- Hence smoking results in increased proteolytic destruction of lung tissue, accelerating the development of emphysema.

Cirrhosis of Liver

α1 Antitrypsin deficiency leads to liver disease:

- GAG to AAG mutation in α 1 AT gene (Mechanism unknown)
- Glu to Lys substitution results in PiZZ Phenotype
- PiZZ accumulate in the cisternae of Endoplasmic Reticulum and aggregates of Liver
- Results in hepatitis leading to Cirrhosis.

GLYCOPROTEINS

Proteins that contain oligosaccharide chains (glycans) covalently attached to their polypeptide backbones. Carbohydrate content is less than 5%.

Biologically Important Glycoproteins[Q]

- Plasma proteins (except albumin)
- Blood group substances
- Hormones (HCG, TSH, LH.FSH[Q])
- Enzyme: Alkaline Phosphatase
- Structural Protein: Collagen
- Transport Proteins: Ceruloplasmin, Transferrin.

Eight Sugars that predominate in Human Glycoproteins are:

- Galactose
- Glucose

- Mannose
- N-Acetylneuraminic acid
- Fucose
- N-Acetylgalactosamine
- N-Acetylglucosamine
- Xylose.

Glycation vs Glycosylation
- *Glycation:* Nonenzymic attachment of sugars to amino group of proteins.
- *Glycosylation:* Enzymic attachment of sugars to protein.

Major Classes of Glycoproteins
- O linked Glycoprotein
- N linked Glycoprotein
- GPI linked Glycoprotein

O-linked Glycoprotein

O-glycosidic linkage between hydroxyl side chain of serine or threonine and N –acetyl galactosamine is present.

Main Features of O-Glycosylation
- O-Glycosylation occurs posttranslationally at certain Ser and Thr residues
- Sugar added in a stepwise manner
- Subcompartments of the Golgi apparatus.
- **Dolichol-P-P-oligosaccharide is not involved.**

N-linked Glycoprotein

N-glycosidic linkage between *amide nitrogen of asparagine and N–acetylglucosamine is present*.

Main Features of N-Glycosylation
- Oligosaccharide Glc3 Man9 (GlcNAc)2 is transferred en bloc from dolichol-P-P-oligosaccharide
- Oligosaccharide: protein transferase, is inhibited by tunicamycin
- Transfer occurs to specific Asn residues
- Occur cotranslationally in the endoplasmic reticulum.

Glycosylphosphatidylinositol-anchored (GPI-anchored, or GPI-linked) Glycoproteins[Q]

Third major class of Glycoproteins.

They link several proteins to Plasma membrane.

Linked to the carboxyl terminal amino acid of a protein via a phosphoryl ethanolamine moiety joined to an oligosaccharide (glycan), which in turn is linked via glucosamine to phosphatidylinositol (PI).

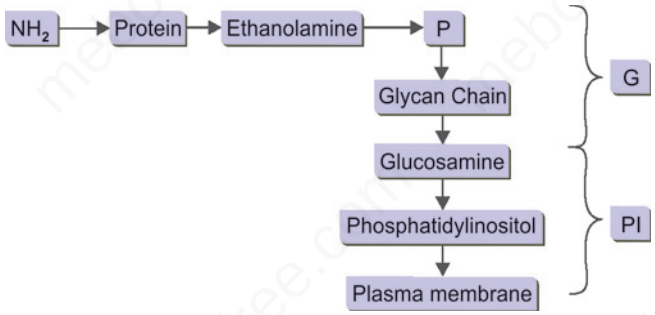


Fig. 2.15: GPI anchored glycoprotein

GPI-Linked Proteins[Q] are:
- Acetylcholinesterase (red cell membrane)
- Alkaline phosphatase (intestinal, placental)
- Decay-accelerating factor (red cell membrane)
- 5′-Nucleotidase (T lymphocytes, other cells).

CLINICAL CORRELATION OF GLYCOPROTEINS

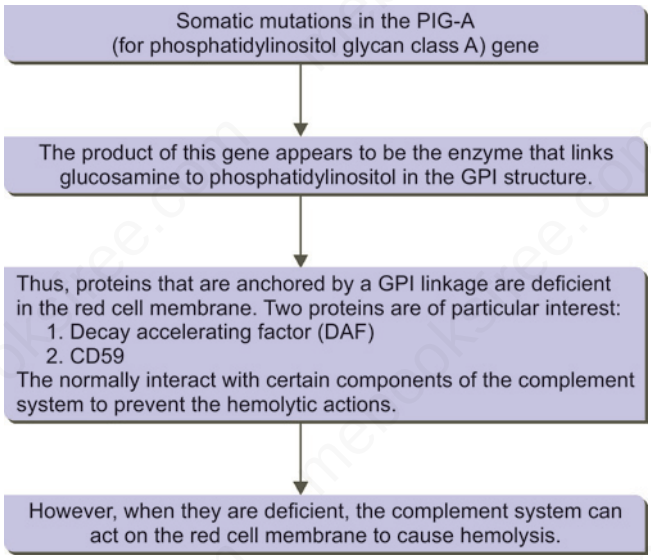
Diseases associated with biosynthesis of Glycoprotein.

Leukocyte Adhesion Deficiency (LAD) II
- Mutations affecting the activity of a Golgi-located GDP-fucose transporter
- The absence of fucosylated ligands for selectins leads to a marked decrease in neutrophil rolling
- Life-threatening, recurrent bacterial infections and also psychomotor and mental retardation
- Oral fucose is the treatment.

Hereditary Erythroblastic Multinuclearity with a Positive Acidified Lysis Test (HEMPAS) or Congenital Dyserythropoietic Anemia Type II
- Abnormalities in certain enzymes α mannosidase II involved in the biosynthesis of N-glycans
- Particularly affecting the red blood cell membrane.

Paroxysmal Nocturnal Hemoglobinuria (PNH)
- Acquired mild anemia characterized by the presence of hemoglobin in urine due to hemolysis of red cells, particularly during sleep
- **Hemolysis occur particularly during sleep in PNH**
- This is due to a slight drop in plasma pH during sleep, which increases susceptibility to lysis by the complement system
- A test done to diagnose PNH is **Hams Test**
- A treatment option is Monoclonal Antibody to C5 (a terminal component of Complement System).

The Basic Defect in PNH



Congenital Muscular Dystrophies (CMDs)

They are:

- Walker-Warburg syndrome
- Muscle-eye-brain disease
- Fukuyama CMD

Defects in the synthesis of glycans in the protein α-dystroglycan (α-DG).

Rheumatoid Arthritis

- Associated with an alteration in the glycosylation of circulating immunoglobulin G (IgG) molecules
- They lack galactose in their Fc regions and terminate in GlcNAc
- Mannose-Binding Protein bind agalactosyl IgG molecules
- Leads to activation of the complement system
- Contributing to chronic inflammation in the synovial membranes of joints.

I Cell Disease

- Mutation in the gene for GlcNAc Phosphotransferase
- Lack of normal transfer of GlcNAc 1-P to mannose residue of enzymes destined to Lysosomes
- Lack of Mannose 6 Phosphate in the enzyme result in defective Protein targeting to Lysosomes.

Congenital Disorders of Glycosylation (CDGs)

- Autosomal recessive disorders
- Multisystem disorders
- Affect the central nervous system, resulting in psychomotor retardation
- At least 15 distinct disorders
- Isoelectric focusing of transferrin is a useful biochemical test for assisting in the diagnosis
- Oral mannose has proved of benefit in the treatment of CDGIa.

ADVANCED GLYCATION END-PRODUCTS (AGES)

- The end-products of glycation reactions are termed advanced glycation end-products (AGEs)
- When glucose attaches to a protein, intermediate products formed include Schiff bases
- These can further re-arrange by the Amadori rearrangement to ketoamines
- The overall series of reactions is known as the Maillard reaction.

Medical Importance of AGEs

- Aging
- Atherogenesis
- Microvascular and macrovascular damage in diabetes mellitus.

Aminoguanidine

- An inhibitor of the formation of AGEs
- Reduce the complication in Diabetes Mellitus.

Glycoproteins Important in Fertilization

- Zonapellucida ZP contains the glycoproteins ZP-3
- ZP3, an O-linked glycoprotein
- Oligosaccharide chains of ZP3 interact specifically with sperm, galactosyl transferase, and induces the acrosomal reaction
- Maybe possible to inhibit fertilization by developing drugs or antibodies that interfere with the normal functions of ZP3 (Contraceptives)

'Sugar Code of Life'

Certain oligosaccharide chains encode **biologic information,** e.g. mannose 6-phosphate residues target newly synthe-sized lysosomal enzymes to that organelle.

PROTEIN SORTING (IMPORTANT FOR AIIMS AND PGI)

Proteins must travel from polyribosomes, where they are synthesized, to many different sites in the cell to perform their particular functions. This process is called Intracellular Traffic of Proteins or simply Protein Sorting.

Remember

Golgi Apparatus is involved in the Glycosylation and Sorting of Proteins.

The sorting decision

- The major decision is at the site of synthesis
- The second by the Signal sequence in the protein.

Site of Synthesis

Two Sorting branches based on the site of synthesis

- Cytosolic Branch
- Rough Endoplasmic Reticulum branch.

Cytosolic Branch (Free Polyribosome)

Proteins synthesized on free polyribosomes lack this particular signal peptide and are delivered to organelle. Signal Sequences direct proteins to specific organelles.

- Mitochondria
- Nuclei
- Peroxisomes
- Cytosol.

Signal Sequences Direct Proteins to Specific Organelles

Target sequence	Organelle targeted
N terminal Signal Peptide Sequence	ER
Carboxy terminal KDEL Sequence (Lys-Asp-Glu-Leu) **HDEL Sequence (His-Asp-Glu-Leu)**	Lumen of ER
Diacidic Sequence (Asp-X-Glu)	Golgi Membranes
Amino terminal Sequence or Matrix Targeting Sequences	Mitochondrial Matrix
Nuclear Localization Sequence (NLS)	Nucleus
Peroxisomal Matrix targeting Sequence (PTS)	Peroxisome
Mannose 6 Phosphate	Lysosome

Rough Endoplasmic Reticulum (RER) Branch

Proteins synthesized on membrane-bound polyribosomes contain a **signal peptide** that mediates their attachment to the membrane of the ER called **Signal Peptide Hypothesis** (explained later).

All the proteins synthesized in membrane bound Polyribosome (RER) destined for various membranes Proteins synthesized in the free ribosomes are targeted to the organelles like:

- Membrane of Endoplasmic Reticulum [ER]
- Membrane of Golgi apparatus [GA]
- Plasma membrane [PM]
- Lysosome

Remember

An exception to proteins synthesized in RER branch is to membranes is proteins destined to Lysosomes, is synthesized in RER

Signal Peptide Hypothesis

- The signal hypothesis was proposed by Blobel and Sabatini
- To explain the distinction between free and membrane-bound polyribosomes
- It is proposed that proteins synthesized on membrane-bound polyribosomes contained an N terminal peptide extension (signal peptide) at their amino terminal which causes them to be attatched to the membranes of ER, and facilitates the protein transfer into the ER lumen
- From ER lumen they are further sorted to various membranes and lysosomes
- Certain ER membrane proteins are transferred directly into the membrane of ER, without reaching the lumen.

Properties of Signal Peptides[Q]

- Located at the amino terminal
- Contain approximately 12–35 amino acids
- Methionine is usually the amino terminal amino acid
- Contain a central cluster of hydrophobic amino acids
- Contain at least one positively charged amino acid near their amino terminal.

ENDOPLASMIC RETICULUM TRANSLOCATION

The transfer of protein across ER membrane into the lumen can be of two types:

Cotranslational Translocation into the Lumen of ER

This transfer occur when the translation is ongoing. These proteins are kept in unfolded state prior to the entry in to conducting channel. The pathway involves a number of specialized proteins and proceeds in 5 steps.

Specialized proteins Involved in Endoplasmic Reticulum Translocation N-terminal signal peptide

- Polyribosomes
- SRP, signal recognition particle (Six proteins associated with an RNA molecule)
- SRP-R, signal recognition particle receptor
- **Translocon[Q] (Sec 61 complex) consist of three membrane proteins, the sec-61 complex that forms the protein conducting channel in the ER.**
- Signal peptidase

Contd...

Contd...

- Associated proteins (e.g. TRAM and TRAP)
- TRAM is Translocating Chain Associated Membrane Protein. TRAM accelerates the translocation of certain proteins
- TRAP, Translocon associated Protein Complex. The function of TRAP is not clear.

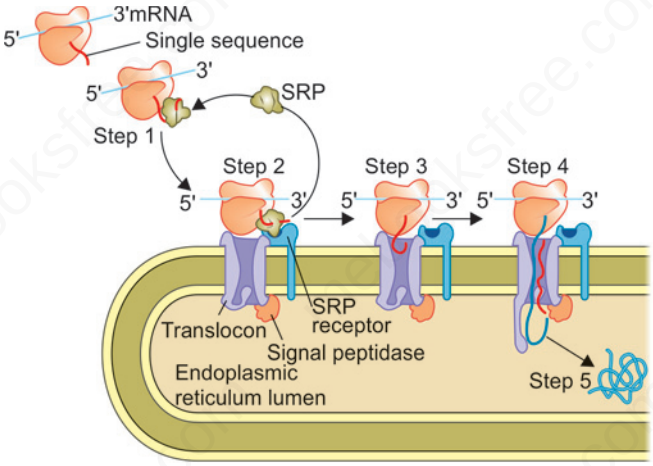


Fig. 2.16: Endoplasmic reticulum translocation-Cotranslational

Steps of Cotranslational Endoplasmic Reticulum Translocation of Proteins (Ref: Figure. 2.8)

Step 1: The signal sequence emerges from the ribosome and binds to the SRP. **This temporarily arrests further elongation of the polypeptide chain (elongation arrest)**Q

Step 2: The SRP–ribosome–nascent protein complex binds to the SRP receptor (SRP-R). SRP guides the protein to SRP-R

Step 3: The SRP is released from SRP-R, the ribosome binds to the **translocon (Sec 61 complex)**, and the signal peptide inserts into the channel in the translocon and translation resumes. **SRP and SRP-R binds to GTP**Q. When SRP to SRP-R interaction occurs GTP is hydrolysed, SRP dissociates from SRP-R

Step 4: The signal peptide induces opening of the channel in the translocon by binding to certain hydrophobic residues in it, thus causing the plug on the translocon to move. The tranlocon opens only when signal peptide is present

Step5: Cleavage of the signal peptide by signal peptidase occurs, and the fully translocated polypeptide/protein is released into the lumen of the ER.

Post-Translational Translocation of Proteins into the lumen of ER

- Less common pathway
- This process involves Sec 61 tranlocon complex, **Sec 62/Sec 63 complex**, **Chaperone protein of hsp-70** family like **BiP** (Binding Immunoglobulin Protein), ATP hydrolysis.

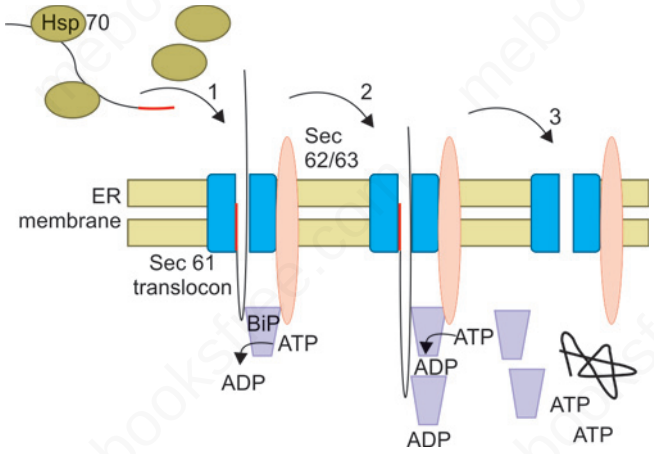


Fig. 2.17: Post-translational translocation of proteins

Steps involved in the post-translational translocation of proteins into the lumen of ER.

- The protein to be translocated initially bind to the translocon
- Cytosolic chaperones are released
- The leading end of peptide bind to the BiP
- ATP bind to BiP interacts with Sec 63/Sec 63
- ATP hydrolysis provide energy to move the protein forwards
- When entire protein has entered into the lumen, ADP is exchanged for ATP, allowing BiP to be released.

Cotranslational Insertion of Proteins into the ER Membrane

Certain proteins inserted into the ER membrane without reaching the lumen.

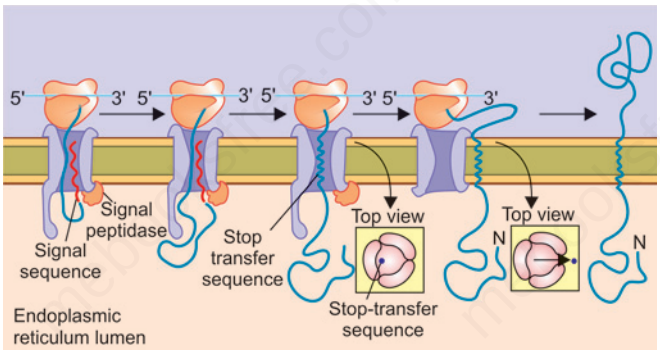


Fig. 2.18: Cotranslational insertion of proteins into the ER membrane

Steps involved in the cotranslational insertion into the ER membrane.

- The proteins partly transverses the ER membrane
- Signal peptide is cleaved

- These proteins contains a highly hydrophobic segment called **halt or stop transfer signal or internal insertion sequence**
- The translocon recognize the stop transfer signal
- This allows the protein to exits the channel via **lateral gate**
- Protein is anchored to the ER membrane.

EXOCYTIC PATHWAY OR SECRETORY PATHWAY OF ROUGH ENDOPLASMIC RETICULUM BRANCH

- This was first delineated by George Palade and colleagues
- The entire pathway of proteins traveling from **ER → GA → Plasma Membrane** is often called Exocytic or Secretory pathway
- The proteins is carried in **vesicles.**

The important vesicles are:

- Transport vesicles
- Secretory Vesicles
- COP I Vesicles
- COP II Vesicles
- Clathrin Coated Vesicles.

Vesicle	Function and characteristics
Transport Vesicle	They carry proteins to Plasma membrane. The transport of protein occur continuously hence called Constitutive ***Secretion***
Secretory Vesicles	Transport of proteins to be secreted out of the cell, like Insulin from β cells of Pancreas. The secretion is regulated by external signals, hence called Regulated Secretion
COP I Vesicle	They carry proteins from Golgi apparatus to the Endoplasmic Reticulum **This is called Retrograde Transport**
COP II Vesicle	They carry proteins from Endoplasmic Reticulum to Golgi Apparatus or Endoplasmic Reticulum – Golgi Intermediate Complex, ERGIC. This is called ***Anterograde transport***
Clathrin coated vesicle	Involved in Endocytosis of proteins from late endosome to Lysosomes. This is called ***Receptor mediated Endocytosis***

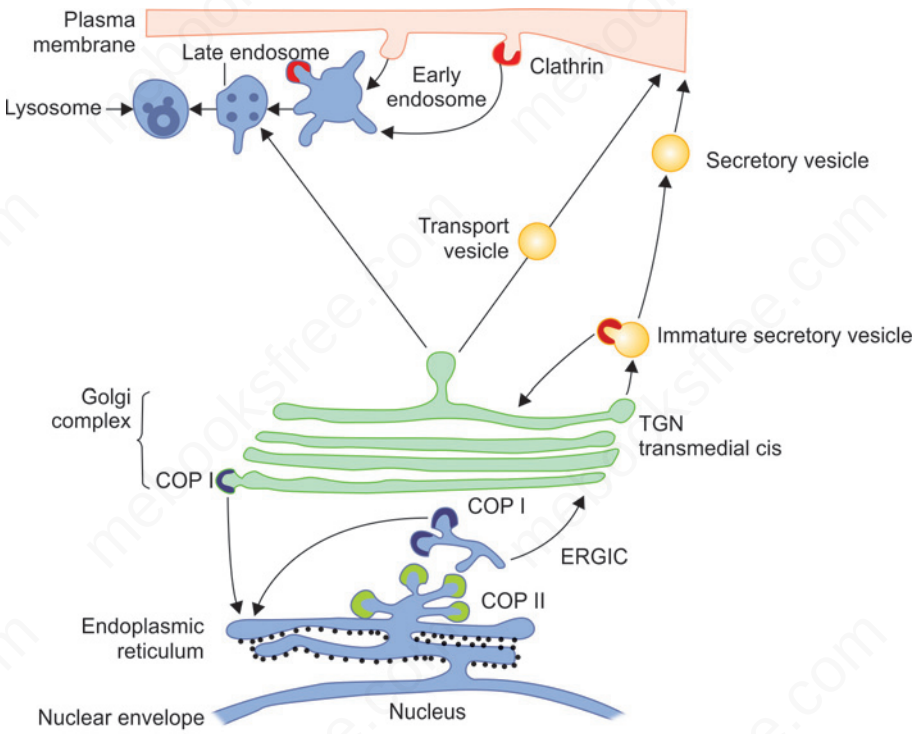


Fig. 2.19: Functions of vesicles in rough endoplasmic reticulum branch of protein sorting

CLINICAL CORRELATION–PROTEIN SORTING

Peroxisome Biogenesis Disorder

- Disorders associated with defect in import of 1 or more proteins to Peroxisome
- Due to mutations in *PEX* genes encoding certain proteins—so-called **peroxins**, involved in various steps of peroxisome biogenesis, such as the import of proteins to peroxisomes.

Peroxisomal Ghosts
Absence or reduction in the number of peroxisomes is pathognomonic for disorders of peroxisome biogenesis. In most disorders, there are membranous sacs that contain peroxisomal integral membrane proteins, which lack the normal complement of matrix proteins; these are peroxisome 'ghosts'.

Due to gene defects, which involve mainly the import of proteins that contain the PTS1 targeting signal

- Zellweger syndrome (Most Severe)
- Neonatal adrenoleukodystrophy
- Infantile Refsum disease (Least Severe).

Due to defect in PEX7, which involves the import of proteins that utilize PTS2

- Rhizomelic chondrodysplasia punctata.

Clinical Picture of Peroxisomal Biogenesis Disorders

Zellweger Syndrome (ZS), Neonatal Adrenoleukodystrophy (NALD), Infantile Refsums Disease (IRD)
These three disorders are considered to form a clinical continuum, with ZS the most severe, IRD the least severe, and NALD intermediate.

Zellweger Syndrome

- Typical facial appearance (high forehead, unslanting palpebral fissures, hypoplastic supraorbital ridges, and epicanthal folds)
- Severe weakness and hypotonia, neonatal seizures
- Eye abnormalities (cataracts, glaucoma, corneal-clouding, Brushfield spots, pigmentary retinopathy, and nerve dysplasia)
- Because of the hypotonia and, mongoloid appearance, Down syndrome may be suspected.

Abnormal laboratory findings common to disorders of peroxisome biogenesis
Peroxisomes absent to reduced in number
Peroxisomal enzyme Catalase in cytosol

Contd...

Contd...

Abnormal laboratory findings common to disorders of peroxisome biogenesis
Deficient synthesis and reduced tissue levels of plasmalogens
Defective oxidation and abnormal accumulation of very long chain fatty acids
Deficient oxidation and age-dependent accumulation of phytanic acid
Defects in certain steps of bile acid formation and accumulation of bile acid intermediates
Defects in oxidation and accumulation of l-pipecolic acid
Increased urinary excretion of dicarboxylic acids

DNA mutations in Peroxisome Biogenesis Disorder

- PEX gene mutation in Zellweger Syndrome, Neonatal Adrenoleukodystrophy, Infantile Refsums Disease
- PEX 7 gene mutation in Rhizomelic chondrodysplasia.

Lysosomal Targeting Disorders

Due to defective targeting of proteins to Lysosomes I (Inclusion) Cell Disease

- Due to lack of targeting signal, Mannose 6 phosphate
- Deficient in the activity of the cis-Golgi-located **GlcNAc phosphotransferase**
- Lack of transfer of GlcNAc 1 P residue to specific mannose residue of certain enzymes destined for Lysosomes
- These enzymes lack Mannose 6 Phosphate
- These enzymes are secreted into plasma rather than to Lysosomes
- Lysozomal enzymes are deficient results in partially digested cellular material, as inclusion bodies
- Clinical picture almost similar to Hurler's Disease.

Psuedo-Hurler Dystrophy

- Closely related to I-cell disease
- It is a milder condition, and patients may survive to adulthood
- The defect in pseudo-Hurler polydystrophy lies in the domain of GlcNAc phosphotransferase that recognizes lysosomal enzymes
- Some catalytic activity is retained.

REVIEW QUESTIONS

Chemistry of Proteins

1. True about Isopeptide bond is: (PGI Nov 2011)

a. It makes protein resistant
b. Bond is formed between carboxy terminal of one protein and amino group of a lysine residue on another
c. Involves in post-transcriptional modification of protein
d. Enzyme act as catalyst for bond formation

Ans. a, b, d

Atypical Peptide Bond (Pseudopeptide Bond) (Isopeptide Bond)

A bond formed between an amino group and a carboxyl group at least one of which is not an alpha group. Seen in the side chains of proteins.

Characteristic Features

- Occur post-translationally
- Can be formed spontaneously or enzymatically
- Can produce stably linked protein dimers, multimers or complexes
- Makes the protein resistant as proteases cannot hydrolyse isopeptide bond.

Examples

- Glutathione
- Thyrotropin Releasing Hormone
- Ubiquitin attached to protein
- Blood clots.

Application of spontaneous Isopeptide Bond formation

- Develop a new peptide tag called Isopeptag. Used in:
- *In vivo* Protein targeting
- Fluorescence Microscopy Imaging.

2. Which of the following is/are storage protein: (PGI May 2011)

a. Myoglobin
b. Ovalbumin
c. Ricin
d. Ferritin
e. Glutelin

Ans. b, d, e (*Ref: Chatterjee and Shinde 8/e p82-85*)

Storage Protein

Proteins that act as store house of amino acids and metal ions that can be easily mobilized.

- Casein of milk
- Vitellin of egg yolk
- Ovalbumin of egg white
- Glutelin of wheat
- Oryzenin of Rice
- Gliadin of Wheat
- Ferritin-stores iron.

Other options

Myoglobin

- It is a transport protein of Oxygen to Skeletal Muscle.

Ricin:

- Inhibitor of mammalian Protein Synthesis
- From castor bean inactivates eukaryotic 28 S ribosomal RNA.

3. Which one of the following can be homologous substitution for isoleucine in a protein in sequence? (AI 2006)

a. Methionine
b. Aspartic acid
c. Valine
d. Arginine

Ans. c. Valine

Conservative [Homologous] Substitution

- One amino acid replaced by another amino acid of similar characteristics
- Examples of homologous substitution is shown in the diagram given below.

Hydrophilic, Acidic	Asp	Glu				
Hydrophilic, Basic	His	Arg	Lys			
Polar, Uncharged	Ser	Thr	Gln	Asn		
Hydrophobic	Ala	Phe	Leu	Ile	Val	Pro

Nonconservative (Nonhomologous) Substitution

- One amino acid replaced by another amino acid of different characteristics.

4. In a mutation if valine is replaced by which of the following would not result in any change in the function of protein: (AIIMS May 02)

a. Proline
b. Leucine
c. Glycine
d. Aspartic acid

Ans. b. Leucine

Homologous substitution or Conservative mutation won't result in any change in function of the proteins examples for homologous substitution.

Hydrophilic, Acidic	Asp	Glu				
Hydrophilic, Basic	His	Arg	Lys			
Polar, Uncharged	Ser	Thr	Gln	Asn		
Hydrophobic	Ala	Phe	Leu	Ile	Val	Pro

Homologs, Orthologs and Paralogs

When two genes share detectable sequence similarities (nucleotide sequence in DNA and amino acid sequence in the proteins they encode), their sequence are said to be **homologous** and the proteins encoded by them are called **homologs.**

Homologs arises from a common ancestor.

Homologs can be orthologs orparalogs.

- Homologous genes in same species, are said to be paralogous and their protein products are paralogs. Paralogous genes are result of gene duplication.
  - Paralogs are similar not only in sequence of amino acids but also in the three dimensional structure.
- Homologous genes in different species, are said to be orthologous and their protein products are orthologs.
  - The sequence difference between homologous gene may be taken as a rough measure of degree to which two species have diverged during evolution.

5. At isoelectric pH protein: (PGI June 03)

a. Have net charge '0'
b. Do not migrate
c. Are positively charged
d. Are negatively charged

Ans. a. Have net charge 0, **b.** Do not migrtate *(Ref: Harper 30/e p20)*

Properties of proteins at isoelctric pH

- They have no net charge
- They do not have electrophoretic mobility
- Maximum precipitability
- Minimum solubility
- Minimum buffering action

6. Proteins are linear polymers of amino acids. They fold into compact structures. Sometimes, these folded structures associate to form homo-or-heterodimers. Which one of the following refers to this associated form? (AI 2006)

a. Denatured state
b. Molecular aggregation
c. Precipitation
d. Quaternary structure

Ans. d. Quaternary structure *(Ref: Harper 30/e p39)*

Primary Structure is the linear sequence of amino acid held together by peptide bonds in its peptide chains. Bond involved in primary structure is Peptide Bond, a type of covalent bond.

Secondary Structure

- Configurational relationship between residues which are about 3–4 amino Acids apart in linear sequence.
- The folding of short (3- to 30- residue), contiguous segments of polypeptide into geometrically ordered units
- Bonds involved in the secondary structure are primarily **noncovalen**t bonds like:
  - Hydrogen Bond (Most important Bond)
  - Hydrophobic Bond
  - Electrostatic Bond (Ionic Bond, Salt Bridges)
  - van der Waals forces.

Tertiary structure: The entire three-dimensional conformation of a polypeptide is referred to as tertiary structure.

Quaternary structure: If more than one polypeptides aggregate to form one functional protein, the spatial relationship between the polypeptide subunits is referred to as quaternary structure.

Bonds involved in Tertiary and Quaternary Structures are primarily noncovalent bonds.

- Hydrophobic Interaction
- Hydrogen Bond
- Electrostatic Bond
- Van der Waal's Forces

7. Isoelectric point is when: (Kerala 2011)

a. Net charge of protein is zero
b. Mass of protein is zero
c. Protein
d. Denaturation of protein occurs

Ans. a. Net charge of a protein is zero *(Ref: Harper 30/e p20)*

8. Biuret test is used for detection of: (Ker 2010)

a. Protein
b. Cholesterol
c. Steroid
d. Sugar

Ans. a. Protein

COLOR REACTIONS OF AMINO ACIDS

Biuret Test

General test for Proteins

Cupric ions in alkaline medium forms violet color with peptide bond nitrogen.

Ninhydrin Test

General test for all alpha amino acid

Amino acid + 2 mols of Ninhydrin → Aldehyde with 1 carbon atom less + CO_2 + Purple Complex (Ruhemann's Purple)

Color Reactions	Test answered by
Xanthoproteic Test **(Conc HNO_3 is a reagent[Q])**	**Aromatic Amino Acid[Q2014DNB]** (Phenyl Alanine, Tyrosine, Tryptophan)
Millon's test	Tyrosine (Phenol)
Aldehyde test can be done in two methods: • Acree Rosenheim Test **Hopkin's Cole Test[Q]**	Tryptophan (Indole group)
Saka **G**uchi's test	Ar**G**inine (**G**uanidinium group) ***Mnemonic–G is common to all***
Sulfur test	Cysteine
Cyanide Nitroprusside Test	Homocysteine
Pauly's Test	Histidine (Imidazole) Tyrosine (Phenol)

9. Which among the following is the structure of myoglobin?

a. Monomer
b. Homodimer
c. Heterodimer
d. Tetramer

Ans. a. Monomer

- Myoglobin is a monomer
- Hemoglobin is a tetramer

10. Denaturation is resisted by which of the following bond ? (NBE pattern[Q])

a. Peptide bond
b. Hydrogen bond
c. Disulfide bond
d. Electrostatic bond

Ans. a. Peptide bond

Denaturation of proteins

- Primary structure is not lost
- Hence peptide bond is not broken
- Secondary and tertiary structure is lost
- There is loss of folding.

11. Polypeptide formation in amino acid is by: (NBE pattern[Q])

a. Primary structure
b. Secondary structure
c. Tertiary structure
d. Quaternary structure

Ans. a. Primary structure *(Ref: Harper 30/e p30)*

Primary Structure: It is the linear sequence of amino acid held together by peptide bonds in its peptide chains. Bond involved in primary structure is Peptide Bond, a type of covalent bond.

Secondary Structure

- Configurational relationship between residues which are about 3–4 amino Acids apart in linear sequence.
- The folding of short (3- to 30-residue), contiguous segments of polypeptide into geometrically ordered units.

Tertiary structure: The entire three-dimensional conformation of a polypeptide is referred to as tertiary structure.

Quaternary structure: If more than one polypeptides aggregate to form one functional protein, the spatial relationship between the polypeptide subunits is referred to as Quaternary structure.

12. Rossman fold associated NADH domain is found in which enzyme: (NBE Pattern[Q])

a. Pyruvate Dehydrogenase
b. Lactate Dehydrogenase
c. Malate Dehydrogenase
d. Isocitrate Dehydrogenase

Ans. b. Lactate Dehydrogenase

Oxidoreductase with Rossmann Fold

- Lactate dehydrogenase
- Alcohol dehydrogenase
- Glyceraldehyde-3-phosphate dehydrogenase
- Malate dehydrogenase
- Quinone oxidoreductase
- 6-phosphogluconate dehydrogenase
- D-glycerate dehydrogenase
- Formate dehydrogenase
- 3 α, 20 β -hydroxysteroid dehydrogenase.

13. In forming 3D structure following factors help: (PGI May 2014)

a. Peptide bond
b. Amino acid sequence
c. Interaction between polypeptide

d. Chaperone
e. Side chain

Ans. b, c, d, e

(Ref: Harper 30/e p39, 40, 43, 44; Stryer 7/e p60)

- Amino Acid sequence determines the tertiary structure of proteins
- Side chains help in the formation of bonds involved in tertiary structure of proteins
- Interaction of polypeptide also helps the three dimensional structure of proteins
- Chaperone helps in protein folding; hence it helps in three dimensional structure.
- But Peptide bond helps in the primary structure.

14. Confirmatory test for proteins are: **(PGI May 2014)**

a. Western Blot
b. ELISA
c. Chip assay
d. Dot blot

Ans. a, b, c, d

Western blot, ELISA, Chip assay and dot blot is based on Antigen antibody interaction. Hence, they are confirmatory test for proteins. Chip is the other name for Microarray. Just like DNA Chip, where DNA–DA Hybridization is done, there Protein Microarray or Protein Chip where Antigen antibody interaction is done.

- Dot blot or slot blot is a blot technique in which the step blotting to nitrocellulose membrane is not done.
- This can be used for proteins also.

15. What type of protein is Casein? **(CMC Ludhiana 2014)**

a. Lipoprotein
b. Phosphoprotein
c. Glycoprotein
d. Flavoprotein

Ans. b. Phosphoprotein

(Ref: Chatterjea and Shinde 8/e p85)

Two important Phosphoproteins are

- Casein found in milk
- Ovovitellin found in egg yolk

16. Which is /are not transport Protein: **(PGI May 2012)**

a. Transferrin
b. Collagen
c. Ceruloplasmin
d. Hemoglobin
e. Albumin

Ans. b. Collagen *(Ref: Harper 30/e p672, table 52-3)*

Plasma Proteins in Transport FunctionQ

Transport Protein	Compound it binds
Albumin	• Bilirubin • Free fatty acids • Ions (Ca^{2+}) • Metals (e.g. Cu^{2+}, Zn^{2+}) • Met Heme • Steroids • Hormones
Ceruloplasmin	• Cu^{2+}
Corticosteroid-binding globulin (transcortin)	• Cortisol
Haptoglobin	• Extra corpuscular hemoglobin
Lipoproteins	• Plasma Lipids
Hemopexin	• Heme
Retinol-binding protein	• Retinol
Sex-hormone-binding globulin	• Testosterone • Estradiol
Thyroid-binding globulin	• T_4 • T_3
Transferrin	• Iron
Transthyretin (formerly prealbumin)	• T_4 and forms a complex with retinol-binding protein

17. Which of the amino acid is responsible for peptide bond: **(PGI Nov 2014)**

a. Amino group
b. Carboxyl group
c. Side chain
d. Amide group
e. Aldehyde group

Ans. a, b

- Peptide bond is formed between amino group of one amino acid with carboxyl group of the next amino acid.

18. Which one of the following about protein structure is correct: **(PGI May 2015)**

a. Protein consisting of one polypeptide can have quaternary structure
b. The formation of disulfide bond in a protein requires that the two participating cysteine residues be adjacent to each other in the primary sequence of the protein
c. The stability of quaternary structure in protein is mainly the result of covalent bonds among the subunits.

d. The denaturation of proteins always leads to irreversible loss secondary and tertiary structure.
e. The information required for the correct folding of a protein is contained in the specific sequence of amino acid along the polypeptide chain.

Ans. e. The information required for the correct folding of a protein is contained in the specific sequence of amino acid along the polypeptide chain.

- Proteins with more than one polypeptide chain can only have quaternary structure.
- The cysteine residues need not be adjacent for the formation of disulfide bond
- The stability of quaternary structure is by noncovalent bonds.
- Denaturation can be reversible also.

19. An alpha helix of a protein is most likely to be disrupted if a missense mutation introduces the following amino acid within the alpha helical structure: (AIIMS Nov 2002)

a. Alanine
b. Aspartic acid
c. Tyrosine
d. Glycine

Ans. d. Glycine *(Ref: Harper 30/e p38)*

- Glycine induces bends in the alpha helix.
- Proline also disrupts the conformation of alpha helix.
- But proline is present in the first turn of alpha helix.

Separatory Techniques of Proteins, Study of Protein Structure, Precipitation of Proteins

20. Precipitation of proteins occur in all except: (AIIMS Nov 2015)

a. Adding alcohol and acetone
b. pH changes is moved away from isoelectric pH
c. With Trichloroacetic acid
d. With heavy metals

Ans. b. pH changes away from isoelectric pH

- Precipitability maximum at isoelectric pH, so pH of medium should be brought to isoelectric pH.

Precipitation reactions of proteins

Methods to precipitate the protein by neutralizing the charge are

Precipitation by heavy metallic salt.

- The reagents used are Mercuric nitrate, Zinc Sulfate, Lead acetate, Ferric Chloride.

Precipitation by Acids.

- Acids bring the pH of the medium to isoelectric pH, precipitability is maximum at isoelectric pH. This is because at isoelectric pH proteins carry no net charge, hence no shell of hydration.
- The reagents used are Phsophotungstic acid, Sulphosalicylic acid, Phosphomolybdic acids, Trichloroacetic acid.

Methods to precipitate protein by removing the shell of hydration

- Precipitation by neutral salts
  - Concentrated Salt solution removes the shell of hydration.
  - Reagents used are Ammonium sulfate. This is called Salting out.
- Precipitaion by organic solvents
  - The reagent used are ether, alcohol, acetone etc.

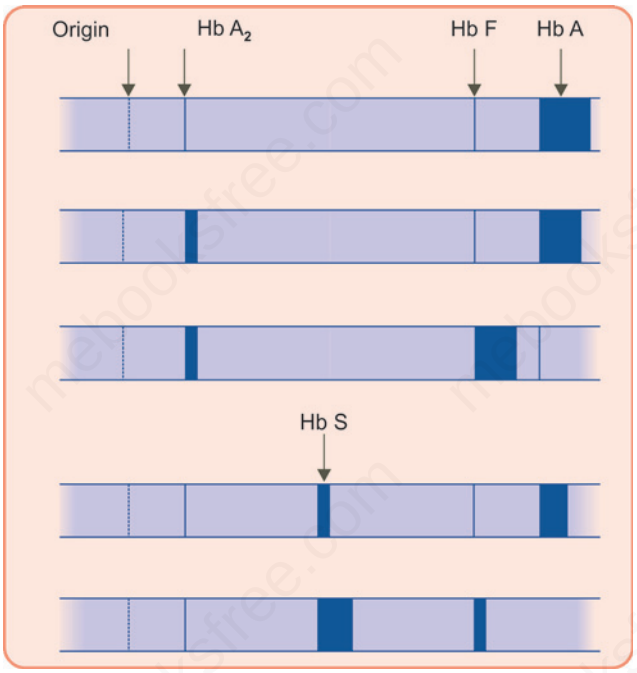
21. In Hb S, Glutamic acid replaced by valine. What will be its electrophoretic mobility? (AIIMS Nov2015)

a. Increased
b. Decreased
c. No change
d. Depends on level of concentration of HbS

Ans. b. Decreased

In Hb S, Glutamic acid is replaced by Valine. Hence the negative charge is decreased. So Hb S moves slower than HbA_1.

The relative mobility of various Hb fractions in Hb electrophoresis.



Relative mobility of common Hb fractions from origin or point of application is

1 HbA_2, 2. HbS, 3. HbF, 4. HbA_1

22. Following SDS PAGE electrophoresis, protein is found to be 100 kDa, After treatment with mercaptoethanol, it shows 2 bands of 20 KDa and 30 KDa widely separated. True statement is:

a. Protein has undergone hydrolysis of S-S linkage
b. It is a dimer of 2 subunits of 20 and 30 KDa
c. It is a tetramer of 2,20 KDa and 2,30 KDa
d. Protein break down due to noncovalent linkage

Ans. c. It is a tetramer of 2,20 KDa and 2,30 KDa

(Ref: Harper 30/e p28)

SDS-PAGE in conjunction with 2 Mercaptoethanol or dithioreitol

- Oxidatively cleave disulfide bond.
- So separate the components of multimeric proteins

Option A—Protein is undergoing oxidative cleavage of S-S bond not hydrolysis

Option B—The protein is 100 KDa. Two subunits of 20 KDa and 30 KDa will not make a 100 KDa protein.

Option D—Disulphide bond is a covalent bond not a noncovalent bond.

23. Protein is purified using ammonium sulfate by: **(AIIMS Nov 2010)**

a. Salting out
b. Ion exchange chromatography
c. Mass chromatography
d. Molecular size exclusion

Ans. a. Salting out *(Ref: Harper 29/e p2, Table 1.1)*

Salt Fractionation (salting out): Protein is purified using ammonium sulfate.

24. All of the following can determine the protein structure, except: **(AIIMS Nov 2008)**

a. High performance liquid chromatography
b. Mass spectrometry
c. X-ray crystallography
d. NMR spectrometry

Ans. a. High performance liquid chromatography

(Ref: Harper 29/e p2, Table 1.1)

Methods for Separating and Purifying Biomolecules

- Salt fractionation (e.g. precipitation of proteins with ammonium sulfate)
- Chromatography: Paper, ion exchange, affinity, thin-layer, gas-liquid, high-pressure liquid, gel filtration
- Electrophoresis: Paper, high-voltage, agarose, cellulose acetate, starch gel, polyacrylamide gel, SDS-polyacrylamide gel
- Ultracentrifugation.

Methods for Determining Biomolecular Structures

- Elemental analysis
- UV, visible, infrared, and NMR spectroscopy
- Use of acid or alkaline hydrolysis to degrade the biomolecule understudy into its basic constituents
- Use of a battery of enzymes of known specificity to degrade the biomolecule under study (e.g. proteases, nucleases, glycosidases)
- Mass spectrometry
- Specific sequencing methods (e.g. for proteins and nucleic acids)
- X-ray crystallography.

25. Method used to study the structure of proteins include all except:

a. UV Spectroscopy
b. NMR Spectroscopy
c. X-ray crystallography
d. Edman's technique

Ans. d. Edman's Technique

(Ref: Harper 29/e p2, Table 1.1)

- Edman's reaction is used to detect sequence of amino acids in a polypeptide.

26. Sanger's reagent is chemically: **(NBE pattern^Q)**

a. 2, 4 Dinitro benzene
b. 2, 4 Dinitro Cresol
c. 1, Flouro 2, 4 Dinitro Benzene
d. 2, 4 Fluoro Dinitro Cresol

Ans. c. 1, Fluoro 2, 4 Dinitro Benzene

- Sanger's reagent is 1 Fluoro2, 4 Dinitro benzene
- Edman's reagent is Phenyl Isothiocyanate

27. All of the following are true about Sickle cell disease, except: **(AI 2008)**

a. Single nucleotide change results in change of Glutamine to Valine
b. RFLP result from a single base change
c. 'Sticky patch' is generated as a result of replacement of a nonpolar residue with a polar residue
d. HbS confers resistance against malaria in heterozygotes.

Ans. c. 'Sticky patch' is generated as a result of replacement of a nonpolar residue with a polar residue Sickle Cell Anemia (HbS)

Molecular basis of Sickle Cell Disease

Point mutation in the sixth codon of β-globin (GAG GUG) that leads to the replacement of a Glutamate (Polar) residue with a Valine (Nonpolar) residue

Investigations

- Hb electrophoresis—Hb S migrate slower in an electric field because less negative due to glu replaced by valine
- Sickling Test
- HPLC to fractionate Hb
- Isoelectric Focussing
- Allele-specific oligonucleotide probe detects hemoglobin (Hb) S allele.
- Direct diagnosis of sickle cell disease using RFLP.

28. True about double diffusion technique is: (NBE pattern[Q])

a. Radial Immunofluorescence
b. Invented by Oakley Fulthrop
c. Ougundi technique
d. Ouchterlony Double immune diffusion

Ans. d. Ouchterlony Double Immune diffusion

(Ref: Tietz Fundamentals of clinical chemistry 5/e p181)

Immunochemical method used for identification of antigens and antibodies

For Qualitative Purposes

Passive Gel Diffusion

- Single Immuno Diffusion:
  - Concentration gradient for only a single reactant. Diffusion of antigen into agar impregnated with antibody. Radial Immune Diffusion is a quantitative method based on Single Immuno diffusion.
- Double Diffusion (Ouchterlony Technique):
  - Concentration gradient is established for both antigen and antibody. It permits comparison of two or more test materials and provides a simple and direct method used to determine whether the antigens in the test specimens are identical, cross reactive, or nonidentical. This technique was named after the Swedish Physicist Orjan Ouchterlony.

Immuno electrophoresis

Technique used to separate and identify the various protein species contained in a sample. This technique is used to study of antigen mixtures and human gammopathies.

Western Blotting

29. Protein separation based on mass/mol wt (size) is/are done in all except: (PGI May 2012)

a. Ultrafiltration
b. Native gel electrophoresis
c. 2D Gel Electrophoresis
d. Gel Filtration Chromatography
e. Ultracentrifugation

Ans. b. Native Gel Electrophoresis

(Ref: Tietz: Fundamentals of Clinical Chemistry 5/e p135)

- Native gel electrophoresis is based on charge
- Ultrafiltration/Ultracentrifugation-based on density, molecular mass
- 2D Electrophoresis based on isolelectric pH and Mol wt (size)
- Gel filtration chromatography/Molecular sieve chromatography is based on Mol wt/size.

30. Method (s) to determine protein structure is/are: (PGI 2014)

a. X-ray Crystallography
b. NMR Spectroscopy
c. Electrophoresis
d. Ultrasonography
e. Infra red spectroscopy

Ans. a, b, e *(Ref: Harper 29/e p2 Table 1.1)*

31. In SDS-PAGE, proteins are separated on basis of: (PGI June 2009)

a. Mass
b. Charge
c. Density
d. Molecular weight
e. Solubility

Ans. a, d *(Ref: Harper 30/e p28)*

PAGE

- Protein is separated based on molecular mass or mol wt/size and charge

SDS PAGE

- SDS impart equal negative charge so that it masks the inherent charge of the Protein
- Now Proteins separate based on Mol wt (Size) only

SDS-PAGE in conjunction with 2 Mercaptoethanol or dithioreitol

- Oxidatively cleave disulfide bond.

So separate the components of multimeric proteins

32. Method of chromatography in which molecules that are negatively charged are selectively released from stationary phase into the positively charged molecules in mobile phase is termed as: (AI 2010)

a. Affinity chromatography
b. Ion-Exchange chromatography
c. Adsorption chromatography
d. Size-Exclusion chromatography

Ans. b. Ion-Exchange chromatography

Chromatography	Stationary phase used	Property used for separation
Paper Chromatography	Water held on a solid support of filter paper (or Cellulose)	Based on the polarity Least Polar moves faster
Thin Layer Chromatography	Silica gel (Kieselguhr) spread on a glass plate or a plastic sheet or aluminium sheet.	Based on Polarity Least Polar moves faster
Ion Exchange Chromatography	Column of Ion exchange resins Anion exchange or Cation exchange resins	Based on Charge-Charge Interaction
Size Exclusion Chromatography Other names Molecular Sieve Chromatography Gel Filtration Chromatography Gel Permeation Chromatography	Column of porous beads	Based on Molecular weight (Size) Particles emerge in the Descending order of Stokes Radius[Q]
Affinity Chromatography[Q]	Column of resins bound to specific ligands used.	Based on specific ligand binding behavior or Biological activity
Hydrophobic Interaction Chromatography		Based on hydrophobic interaction.
Absorption Chromatography		Based on absorption property.

33. Movement of protein from nucleus to cytoplasm can be seen by? (AIIMS 2010)

a. FISH
b. FRAP
c. Confocal microscopy
d. DNA microscopy

Ans. b. FRAP

FRAP is a technique used to study Fluid mosaic model of cell membrane, movement of proteins, etc. FRAP is Fluorescence Recovery After Photobleaching

The technique

Fluorescent dyes emit colored light when it is illuminated, but if a very high intensity light is used then these dyes are unable to fluorescence, other wise called photobleached. Later it recover fluorescence

This recovery after photobleaching is used to study movement of proteins lipids carbohydrates, etc.

34. Amino acid sequence is not found by: (NBE pattern[Q])

a. Sanger's reagent
b. Benedicts reagent
c. Trypsin
d. Cyanogen bromide

Ans. b. Benedict's reagent *(Ref: Harper 30/e p31, 32)*

Methods of Protein Sequencing

Sanger's Technique

- Sanger's Reagent is 1, Fluoro 2, 4 Dinitrobenzene[Q]
- Sanger's Reagent derivatizes the amino terminal residues.
- The first Protein to be sequenced by the method is Insulin by Fredrick Sanger. He got Nobel prize in 1958.
- *Only dipeptides or tripeptides can be sequenced.*

Edman's Technique

- By using Edman's Reagent (Phenyl Iso-Thio-cyanate).
- Phenyl Isothiocyanate derivatizes the amino terminal of Polypeptide.
- Edman's Technique can sequence many residues (5–30 residues) of a single polypeptide sample unlike Sanger's

35. Molecules up to size 4 KD is identified by:

a. Gene array chip
b. Electron spray ionization
c. Quadrupole mass spectrometry
d. Matrix assisted Laser Desorption ionization

Ans. c. Quadrupole mass spectrometry

(Ref: Harper 30/e p31, 32)

Quadrupole mass spectrometers generally are used to determine the masses of molecules of 4000 Da or less, whereas time-of-flight mass spectrometers are used to determine the large masses of complete proteins.

36. Protein fragment separation is/are done by:

a. Western blot
b. Chromatography
c. Centrifugation
d. Ultrafiltration
e. Electrophoresis

Ans. b, c, d, e

- Chromatography, Centrifugation, Ultrafiltration, electrophoresis are separatory techniques
- Western blot is method to detect protein by Antigen antibody interaction.

Fibrous Proteins

37. Collagen of which type is found in hyaline cartilage: (AIIMS Nov 2007)

a. Type I
b. Type II
c. Type III
d. Type IV

Ans. b. Type II *(Ref: Harper 30/e p628)*

Types of Collagen

- Major Collagen present in Bone-Type I (90%)
- Major Collagen present I Dermis, ligaments and tendons-Type I (80%)
- Major Collagen present in Cartilage-Type II (40–50%)
- Major Collagen present in Hypertrophic Cartilage-Type X
- Major Collagen present in Aorta-Type I and Type III (20–40% each)
- Major Collagen present in Basement Membrane Type IV
- Most abundant Collagen-Type I
- Major Collagen in Keloid is Type 3 >> Type 1

Collagen in Wound Healing[Q]

At first a provisional matrix containing fibrin, plasma fibronectin, and type III collagen is formed, but this is replaced by a matrix composed primarily of type Icollagen

38. True about Collagen (PGI May 2011)

a. Triple helix
b. β pleated structure
c. Vitamin C is necessary for post-translation almodification
d. Glycine residue at every third position

Ans. a, c, d *(Ref: Harper's 30/e p629)*

Structure of Collagen

Characteristic Features

Glycine-X-Y repeats

Every third amino acid residue in collagen is a glycine residue.

Alpha Chain

Polyproline helix of three residues per turn twisted in left-handed direction. Each polypeptide chain contains 1000 Amino Acids.

Triple helical structure

Three of these alpha chains are then wound into a right-handed super helix.

Quarter Staggered arrangement

Lateral association of the triple helical units

Each is displaced longitudinally from its neighbor by slightly less than one-quarter of its length. Responsible for tensile strength of Collagen Fibers

39. Keratin is present in both skin and nail. But nail is harder than skin. The reason is: (AI 2012)

a. Increased no of disulfide bonds
b. Decreased no of water molecules
c. Increased Na content
d. Increased hydrogen bonds

Ans. a. Increase in disulfide bond.

- The more the disulfide bond, the harder the protein.
- Keratin is rich in cysteine.

40. The structural proteins are involved in maintaining the shape of a cell or in the formation of matrices in the body. The shape of these protein is: (AI 2006)

a. Globular
b. Fibrous
c. Stretch of beads
d. Planar

Ans. b. Fibrous *(Ref: Vasudevananr Sreekumari 7/e p43)*

Based on the shape of protein are classified into:

1. Fibrous Protein

- Elongated or Needle shaped or long cylindrical or rod like
- Minimum Solubility in water
- Regular Secondary Structure
- Axial Ratio>10
- They are Structural Proteins
- Eg: Collagen, Elastin, Keratin

2. Globular Proteins

- Spherical or oval or Spheroidal in shape
- Easily Soluble
- Axial Ratio < 3
- They perform dynamic functions, e.g. Albumin, Globulin, most enzymes

41. Quarter staggered arrangement is seen in:

a. Immunoglobulin
b. Hemoglobin
c. Collagen
d. Keratin

Ans. c. Collagen *(Ref: Harper 30/e p629)*

- Triple Helix in Collagen
- Quarter Staggered arrangement in Collagen
- Covalent cross links in collagen
- Desmosine cross links in elastin

42. All of the following are required for hydroxylation of proline in collagen synthesis except: (AI 1997)

a. O_2
b. Vitamin
c. Monoxygenases
d. Pyridoxal phosphate

Ans. d. PLP *(Ref: Harper 30/e p628)*

- Prolyl and Lysyl Hydroxylases are Monooxygenases, require molecular O_2, Vitamin C and, α Keto Glutarate.

43. Major type of collagen in basement membrane: (AIIMS May 2015)

a. Type I
b. Type II
c. Type III
d. Type IV

Ans. d. Type IV *(Ref: Harper 30/e p628)*

Key points distribution of collagen

- Type of collagen present in the noncartilaginous connective tissue, like bone, tendon: Type I
- Type of collagen present in the Cartilage: Type II
- Type of collagen present in the vitreous humor: Type II
- Type of collagen present in the basement membrane-mainly: Type IV (rarely Type XIX)
- Type of collagen present in the dermal epidermal junction: Type VII
- Type of collagen present in the extensible connective tissue like skin, lung, vascular system: Type III
- Type of collagen present in the hypertrophic cartilage: Type X
- Type of collagen present in the skin hemidesmoses: Type XVII
- Type of collagen present in the rhabdomyosarcoma cell: Type XIX
- Type of collagen present in the brain: Type XXV
- Type of collagen present in the testis and ovary: Type XXVI

Type	Distribution	Type	Distribution
I	Noncartilaginous connective tissues, including bone, tendon, skin	XV	Associated with collagens close to basement membranes in many tissues including in eye, muscle, microvessels
II	Cartilage, vitreous humor	XVI	Many tissues
III	Extensible connective tissues, including skin, lung, vascular system	XVII	Epithelia, skin hemidesmosomes
IV	Basement membranes	XVIII	Associated with collagens close to basement membranes, close structural homologue of XV
V	Minor component in tissues containing collagen I	XIX	Rare, basement membranes, rhabdomyosarcoma cells
VI	Muscle and most connective tissues	XX	Many tissues, particularly corneal epithelium
VII	Dermal-epidermal junction	XXI	Many tissues
VIII	Endothelium and other tissues	XXII	Tissue junctions, including cartilage-synovial fluid, hair follicle-dermis
IX	Tissues containing collagen II	XXIII	Limited in tissues, mainly transmembrane and shed forms
X	Hypertrophic cartilage	XXIV	Developing cornea and bone
XI	Tissues containing collagen II	XXV	Brain
XII	Tissues containing collagen I	XXVI	Testis, ovary
XIII	Many tissues, including neuromuscular junctions and skin	XXVII	Embryonic cartilage and other developing tissues, cartilage in adults
XIV	Tissues containing collagen I	XXVIII	Basement membrane around Schwann cells

Protein Sorting, Glycoprotein

44. Proteins are sorted by: (AI 2009)

a. Golgi Bodies
b. Mitochondria
c. Ribosomes
d. Nuclear Membrane

Ans. a. Golgi bodies *(Ref: Harper 30/e p608)*

Functions of Golgi bodies

- O-Glycosylation of proteins
- Protein sorting
- Processing of oligosaccharide chains of Glycoproteins

45. Not true among the following is: (PGI 05)

a. Sec 61 translocon complex form passage way
b. SRP-R is a docking protein
c. SRP blocks elongation
d. SRP-R releases elongation block
e. SRP-R is ATP bound

Ans. e. SRP-R is ATP bound

- **SRP**-R, signal recognition particle receptor is a docking protein
- Translocon[Q] (Sec 61 complex) consist of three membrane proteins, the sec-61 complex that forms the protein conducting channel in the ER.
- The signal sequence emerges from the ribosome and binds to the SRP. **This temporarily arrests further elongation of the polypeptide chain (elongation arrest).**[Q]
- **SRP and SRP-R binds to GTP**[Q] not ATP
- The SRP is released from SRP-R translation resumes

Steps of Co translational Endoplasmic Reticulum Translocation of Proteins
Step 1: The signal sequence emerges from the ribosome and binds to the SRP. **This temporarily arrests further elongation of the polypeptide chain (elongation arrest).**[Q]
Step 2: The SRP–ribosome–nascent protein complex binds to the SRP receptor (SRP-R). SRP guides the protein to SRP-R
Step 3: The SRP is released from SRP-R, the ribosome binds to the **translocon (Sec 61 complex)**, and the signal peptide inserts into the channel in the translocon and translation resumes. **SRP and SRP-R binds to GTP**[Q]. When SRP to SRP-R interaction occurs GTP is hydrolyzed, SRP dissociates from SRP-R
Step 4: The signal peptide induces opening of the channel in the translocon by binding to certain hydrophobic residues in it, thus causing the plug on the translocon to move. The translocon opens only when signal peptide is present.
Step 5: Cleavage of the signal peptide by signal peptidase occurs, and the fully translocated polypeptide/protein is released into the lumen of the ER.

46. Endoplasmic reticulum–signal transduction is through: (NBE pattern[Q])

a. Translocon
b. Chaperone
c. Ubiquitin
d. Mannose 6 Phosphate

Ans. a. Translocon *(Ref: Harper 29/e p555, 556)*

The singal hypothesis proposed that the protein is inserted into the ER membrane at the same time as its mRNA is being translated on polyribosomes, so-called cotranslational insertion.

Principal Components involved in Endoplasmic Reticulum Translocation

N-terminal signal peptide

- Polyribosomes
- SRP, signal recognition particle
- SR, signal recognition particle receptor
- Sec 61, the translocon[Q]
- Signal peptidase
- Associated proteins (e.g, TRAM and TRAP)
- TRAM is Translocating Chain Associated Membrane Protein. TRAM accelerates the translocation of certain proteins.
- TRAP, Translocon –associated Protein Complex. The function of TRAP is not clear.

47. Not a function of endoplasmic reticulum: (NBE pattern[Q])

a. Protein synthesis
b. Muscle contraction
c. Protein Sorting
d. Glycoproteins

Ans. b. Muscle Contraction *(Ref: Harper 30/e p608)*

- Rough Endoplasmic reticulum is involved in protein synthesis
- N linked glycoproteins are glycosylated in the EPR
- RER along with GA is a part of exocytic pathway of protein sorting

48. Enzyme deficient in I cell Disease: (NBE pattern[Q])

a. GlcNAcphosphotransferase
b. Mannose Phosphotransferase
c. Phospho Diesterase
d. Mannose 6 Phosphate Transferase

Ans. a. GlcNAcphosphotransferase

Inclusion (I) cell disease

- Lysosomal enzymes targeting disorder

- Recognition marker of Lysosomal enzymes is Mannose 6 Phosphate
- I cell disease lack the golgi located NAcetyl Glucosamine phosphotransferase.

49. Targeting sequence that direct Endoplasmic Reticulum resident protein in retrograde flow to ER in COP-I vesicles.

a. KDEL
b. KDAL
c. DALK
d. KDUL

Ans. a. KDEL *(Ref: Harper 30/e p608)*

Retrograde Transport from the GA

A number of proteins possess the amino acid sequence **KDEL** (Lys-Asp-Glu-Leu) at their carboxyl terminal.

KDEL-containing proteins first travel to the **GA** in COPII transport vesicles and interact there with a specific KDEL receptor protein, which retains them transiently. They then **return in COPI transport vesicles to the ER,** where they dissociate from the receptor, and are thus retrieved. HDEL sequences (H = histidine) serve a similar purpose. The above processes result in net localization of certain soluble proteins to the ER lumen.

SEC61 gene, which encodes a channel through which secretory proteins under construction pass into the endoplasmic reticulum lumen

50. Secretory proteins are synthesized in: (AIIMS Nov 05)

a. Cytoplasm
b. Endoplasmic reticulum
c. First in cytoplasm and then in Endoplasmic Reticulum
d. First in endoplasmic reticulum and then in cytoplasm

Ans. b. Endoplasmic reticulum

Proteins are synthesized in the Endoplasmic reticulum and free ribosome.

Protein Folding

51. Which is not a protein misfolding disease?

a. Prion Disease
b. Alzheimer's Disease
c. Beta Thalassemia
d. Ehlers-Danlos Syndrome

Ans. d. Ehlers-Danlos Syndrome *(Ref: Harper 30/e p609)*

The Prion Related Protein Diseases

Prion like changes underlie many neurodegenerative diseases like Protein rich in α helix changes to protein rich in β sheet.

- Alzheimers Disease
- Parkinson's Disease
- Huntington's Disease
- Fronto Temporal Dementia
- Dementia with Lewy Bodies
- Amyloidosis
- Beta thalassemia.

52. Which of the following groups of proteins assist in the folding of other proteins? (AI 2009)

a. Proteases
b. Proteosomes
c. Templates
d. Chaperones

Ans. d. Chaperones *(Ref: Harpers 30/e p609)*

Molecular Chaperones are:

- Hsp 70
- Hsp 90
- Hsp 40 [Cochaperone]
- BiP [Immunoglobulin heavy chain binding protein]
- Glucose Regulated Protein [GRP-94]
- Calreticulin
- Calnexin.

Enzymes assist folding are:

- Protein Disulphide Isomerase
- Peptidylprolylisomerase.

53. All are TRUE about chaperones, except: (Kerala 2012)

a. Many of them are known as heat shock proteins
b. They use energy during the protein-chaperone interaction
c. Ubiquitin is one of the most important chaperone
d. They are present in wide range of species from bacteria to human

Ans. c. Ubiquitin is one of the most important chaperone *(Ref: Harpers 30/e p609)*

Protein Folding

Proteins are conformationally dynamic molecule that can fold into functionally competent conformation

Auxillary Proteins assist Protein Folding, they are called Chaperones.

Properties of Chaperone Proteins

- Present in a wide range of species from bacteria to humans.
- Many are so-called heat shock proteins (Hsp).
- Are inducible by conditions that cause unfolding of newly synthesized proteins (e.g. elevated temperature and various chemicals).

- They bind to predominantly hydrophobic regions of unfolded proteins and prevent their aggregation.
- They act in part as a quality control or editing mechanism for detecting misfolded or otherwise defective proteins.
- Most chaperones show associated ATPase activity.

Ubiquitin is a key Molecule in Protein Degradation

Ubiquitin

- Key molecule in Protein degradation.
- Small protein with 76 Amino Acids.
- Highly conserved protein.
- Attachment of Ubiquitin to Protein to be degraded is called Kiss of Death.
- Ubiquitin bind to the ε amino group of Lysine of the target protein hence it is a Pseudopeptide or Isopeptide Bond.
- Minimum of four Ubiquitin molecules must be attached to commit target molecule to degradation.

54. Amyloid protein in human being is: **(Kerala 2012)**

a. A naturally present protein in normal individuals
b. Involves selectively blood vessels
c. Is visible by naked eyes as whitish cheesy material
d. A material which gets deposited in extra-cellular spaces

Ans. d. A material which gets deposited in extracellular spaces

(Ref: Robbins and Cotran Pathologic basis of Disease 9/e Chapter 6)

Amyloid Fibrils

Physical Nature of Amyloid

- X-ray crystallography and infrared spectroscopy demonstrate a characteristic **cross-β pleated sheet conformation.**
- Congo red staining shows apple-green birefringence under polarized light.
- By electron microscopy amyloid is seen to be made up largely of continuous, nonbranching fibrils with a diameter of approximately 7.5 to 10 nm.

Chemical Nature of Amyloid

- 95% of the amyloid material consists of fibril proteins,
- 5% of the amyloid material consists of P component and other glycoproteins.

55. True about Chaperones: **(PGI May 2012)**

a. Belong to heat shock Proteins
b. Wide range of Expression
c. Present from bacteria to human
d. Ubiquitin is the most important chaperones
e. Also known as Stress Proteins

Ans. a, b, c, e *(Ref: Harper 30/e p559, Table 49.2)*

- Ubiquitin is the key molecule of protein degradation.

56. Which of the following amino acid can have o-Glycoxylation linkage in oligosaccharide molecule: **(AI 2012)**

a. Asparagine
b. Glutamine
c. Serine
d. Cysteine

Ans. c. Serine *(Ref: Harper 30/e p573)*

Major Classes of Glycoproteins

O-linked Glycoprotein

- O-glycosidic linkage between hydroxyl side chain of **serine or threonine** and N-acetylgalactosamine is present

N-linked Glycoprotein

- N-glycosidic linkage between amide nitrogen of **asparagine** and N–acetylglucosamine is present.

Glycosylphosphatidylinositol-anchored (GPI-anchored, or GPI-linked) glycoproteins

- Third major class of Glycoproteins.
- They link several proteins to Plasma membrane.
- Linked to the carboxyl terminal amino acid of a protein via a phosphorylethanolamine moiety joined to an oligosaccharide (glycan), which in turn is linked via glucosamine to phosphatidylinositol (PI).

57. Immunoglobulins are: **(NBE pattern Q)**

a. Proteins
b. Glycoproteins
c. Proteoglycan
d. Glycoside

Ans. b. Glycoproteins

Biologically Important Glycoproteins[Q]

- Plasma proteins (except albumin)
- Blood group substances
- Hormones (HCG, TSH, LH, FSH[Q])
- Enzyme-Alkaline Phosphatase
- Structural Protein-Collagen
- Transport Proteins-Ceruloplasmin, Transferrin.

3 Enzymes

Topics Included

- General Enzymology
- Clinical Enzymology

GENERAL ENZYMOLOGY

Enzymes

Enzymes are highly specialized proteins that can act as catalyst in biological systems. The word enzyme was coined by Frederick Kuhne meaning 'in Yeast'

- *Substrate:* The substance on which enzyme act
- *Product:* The substance produced by the action of enzyme on the substrate. Enzymes are heat labile, thermolabile and proteinaceous in nature
- Exception to proteinaceous nature of enzymes are Ribozymes.

Ribozyme[Q] is RNA with catalytic activity, ***e.g. Sn RNA in Spliceosome***

- Ribonuclease P
- Peptidyl Transferase
- RNase H

Abzyme are antibodies with catalytic activity

Enzymes can be of two types:

1. Simple Enzyme: Consists of only proteins.
2. A complex enzyme consists of:
   - Protein part: Apoenzyme
   - Nonprotein part

Apoenzyme + Nonprotein part (Prosthetic group/Cofactor/ Coenzyme) = Holoenzyme

Nonprotein part can be

- Coenzyme
- Cofactor
- Prosthetic group.

Coenzyme

Thermostable, low molecular weight, nonprotein organic substances are called Coenzymes. A coenzyme can bind covalently or noncovalently to the enzymes. If covalently bound then it is called Prosthetic group. Involvement of coenzyme with substrate is so intimate that, coenzyme is called co-substrates.[Q]

Examples of Coenzymes

Enzyme	Coenzyme
Kinases	ATP/GTP
Dehydrogenases	NAD+/FAD
Pyruvate Dehydrogenase, Alpha Keto Dehydrogenase, Branched Chain Ketoacid Dehydrogenase	TPP, Lipoic Acid, CoA, FAD, NAD+
Transketolase	Thiamine Pyrophosphate
Transaminase	Pyridoxal Phosphate
Carboxylases	Biotin

Cofactor and Prosthetic Group

Prosthetic groups are nonprotein part tightly integrated to the enzyme structure by covalent forces.

- Metals are the most common prosthetic group
- Enzymes which have tightly bound to metal is called metalloenzyme.

Cofactors associate reversibly with Enzymes or Substrates.

- The most common cofactors are also metals
- Enzymes which have metal as cofactors, e.g. loosely bound to them are termed Metal activated Enzymes.

Metal as Cofactors and Prosthetic Group

Metal	Enzymes
Zinc[Q]	Carbonic anhydrase[Q], Carboxypeptidase, Alcohol Dehydrogenase[Q], Alkaline Phosphatase, ALA Dehydratase Lactate Dehydrogenase
Magnesium	Phosphotransferase, (Hexokinase, Phospho-fructokinase) Mutase, Enolase, Glucose 6 Phosphatase
Copper	Tyrosinase, Cyt C Oxidase, Lysyl Oxidase, Superoxide Dismutase, Amino Acid Oxidase
Molybdenum	Xanthine Oxidase, Sulfite Oxidase
Manganese	Enolase, Arginase, Phosphotransferase (Hexokinase, Phosphofructokinase) Mitochondrial Super Oxide Dismutase
Iron	Succinate Dehydrogenase
Calcium	Lipase, Lecithinase

IUBMB CLASSIFICATION–ENZYME CLASSES

As per this system of nomenclature, Enzymes are classified into six main classes:

Enzyme Class No	Enzyme class	Type of reaction catalyzed with examples
I	**Oxidoreductases**	Enzymes that catalyze oxidation of one substrate with reduction of another substrate (described later)
II	**Transferases**	Transfer functional group other than hydrogen from one substrate to another. • Kinases • Transaminase • Transmethylase
III	**Hydrolases**	Enzymes that catalyze hydrolytic cleavage of C—C, C—O, C—N and other covalent bonds. • Lipase • Arginase • Pepsin • Esterases
IV	**Lyases**	Enzymes that catalyze cleavage of C—C, C—O, C—N and other covalent bonds by atom elimination, generating double bonds. • Aldolase • Fumarase • HMG CoA Lyase • Argininosuccinase

Contd...

Contd...

Enzyme Class No	Enzyme class	Type of reaction catalyzed with examples
V	**Isomerases**	Enzymes that catalyze geometric or structural changes within a molecule. • Phosphohexose isomerase • Mutase • Racemase
VI	**Ligases**	Enzymes that catalyze the joining together (ligation) of two molecules in reactions coupled to the hydrolysis of ATP. • Acetyl CoA carboxylase Arginosuccinate synthetase • PRPP synthetase • Carbamoyl phosphate Synthetase • Glutamine synthetase

Oxidoreductases[Q]

Subclassified into

- Dehydrogenase
- Oxygenase
  - Monooxygenase
  - Dioxygenase
- Oxidase
- Catalase
- Peroxidase.

Dehydrogenase

- Catalyze removal of hydrogen from the substrate
- They cannot use oxygen as a hydrogen as the acceptor.

Examples are:

- Alcohol Dehydrogenase
- Lactate Dehydrogenase
- Succinate Dehydrogenase.

Oxidases

- Removal of hydrogen from a substrate with oxygen as the acceptor of Hydrogen.

Examples are:

- Mono Amino Oxidase
- Cytochrome C Oxidase
- Xanthine Oxidase.

Oxygenases

- *Catalyze the direct transfer and incorporation of oxygen into a substrate molecule.*

Monooxygenases [Mixed Function Oxidases or Hydroxylase]

Incorporate one atom of molecular oxygen into the substrate. Examples are:

- Phenylalanine Hydroxylases
- Tyrosine Hydroxylase
- Tryptophan Hydroxylase
- 7 alpha Hydroxylases
- Cytochrome p450.

Dioxygenases

Incorporate both atoms of molecular Oxygen into the substrate. The basic reaction is shown below:

$A + O_2 \rightarrow AO_2$

Examples of Dioxygenase

- Homogentisate oxidase
- Tryptophan Pyrrolase (Dioxygenase)
- Nitric Oxide Synthase.

MECHANISM OF ENZYME ACTION

Explained by different theories:

- Lowering of activation energy
- Michaelis–Menten theory
- Fischer's Template theory
- Koshland's Induced Fit theory.

Lowering of Activation Energy

Enzymes lower the activation energy. Activation energy is defined as the energy required to convert all molecules of a reacting substance from ground state to transition state.

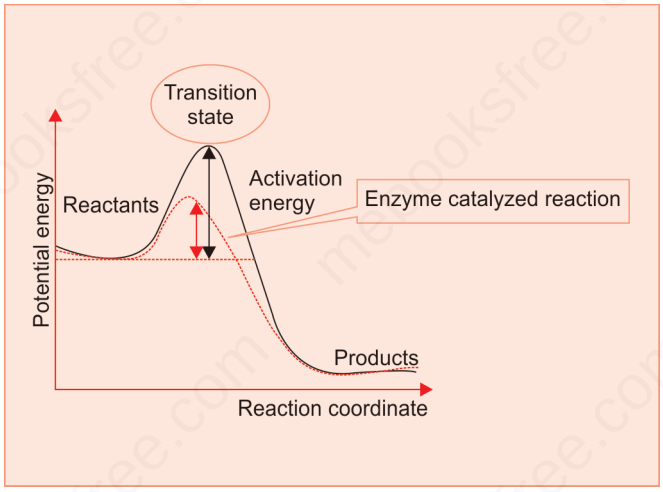


Fig. 3.1: Lowering of activation energy

Michaelis–Menten Theory (Enzyme–Substrate Complex Theory)

Enzyme combines with a substrate to form a transient Enzyme-Substrate Complex which immediately break into Enzyme and products.

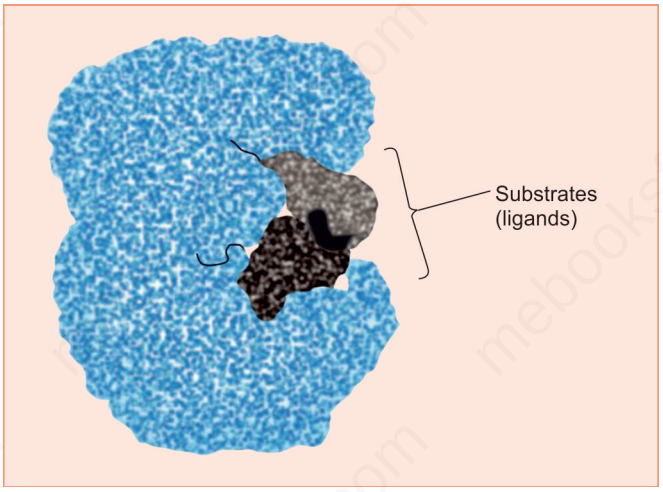


Fig. 3.2: Enzyme substrate complex

Fischer's Template Theory

- Lock and key model
- Three dimensional structure of the active site of unbound enzyme is complementary to the substrate
- Thus Enzyme and substrate fit each other
- Failed to explain dynamic changes that accompany catalysis.

Koshland's Induced Fit Theory

- Binding of substrate to specific part of enzyme induce conformational changes in the active site of the enzyme
- Enzyme changes shape during or after binding with the substrate
- Can explain the dynamic changes that accompany catalysis.

FACTORS AFFECTING ENZYME ACTIVITY

Temperature

- Raising the temperature increases the rate of both uncatalyzed and enzyme-catalyzed reactions by increasing the kinetic energy and the collision frequency of the reacting molecules
- **Bell shaped curve** is obtained by plotting temperature against velocity of reaction
- The optimum temperature for most human enzymes is between 35 and 40°C

- Human enzymes start to denature at temperatures above 40°C
- Enzymes from humans generally exhibit stability at temperatures up to 45–55°C.

Temperature Coefficient (Q10)
The temperature coefficient (Q10) is the factor by which the rate of a biologic process increases for a 10°C increase in temperature. For the temperatures over which enzymes are stable, the rates of most biological processes typically double for a 10°C rise in temperature (Q10 = 2)

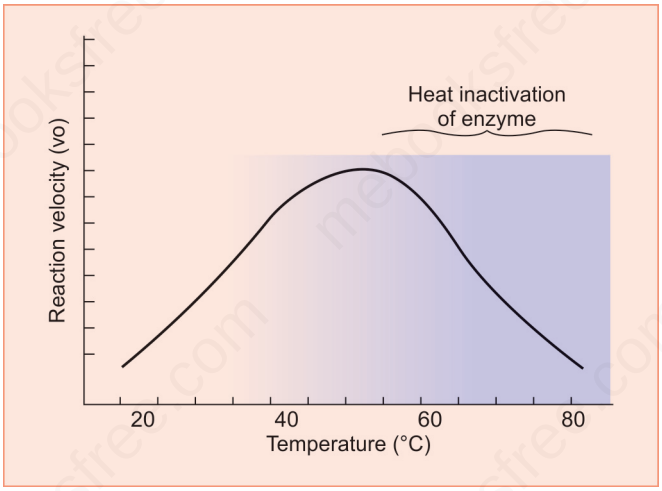


Fig. 3.3: Effect of temperature

Hydrogen Ion Concentration

- The rate of almost all enzyme-catalyzed reactions exhibits a significant dependence on hydrogen ion concentration
- Most intracellular enzymes exhibit optimal activity at pH values between 5 and 9
- The relationship of enzyme activity to hydrogen ion concentration gives a bell shaped curve.

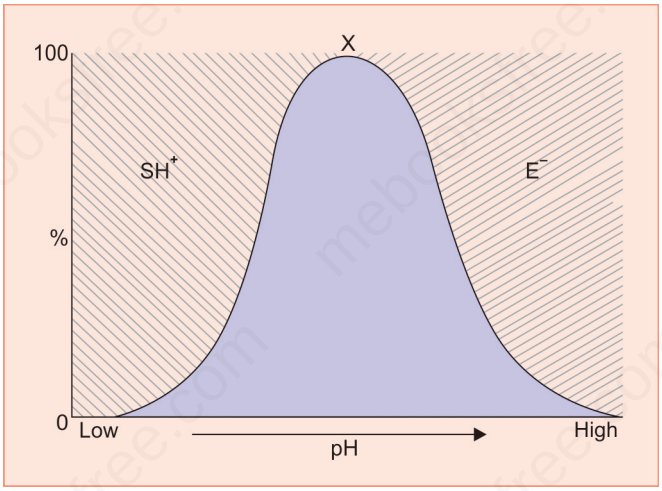


Fig. 3.4: Effect of pH

Enzyme Concentration

In the beginning velocity of enzyme reaction is directly proportional to the enzyme concentration.

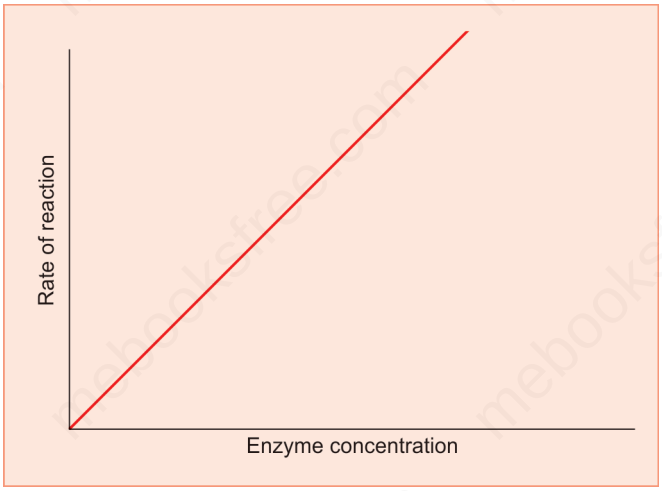


Fig. 3.5: Effect of enzyme concentration

Substrate Concentration

- For a fixed Enzyme concentration, rate of reaction is directly proportional to the substrate concentration up to certain concentration of substrate, but later there is no further increase in velocity
- Hyperbolic curve is obtained.

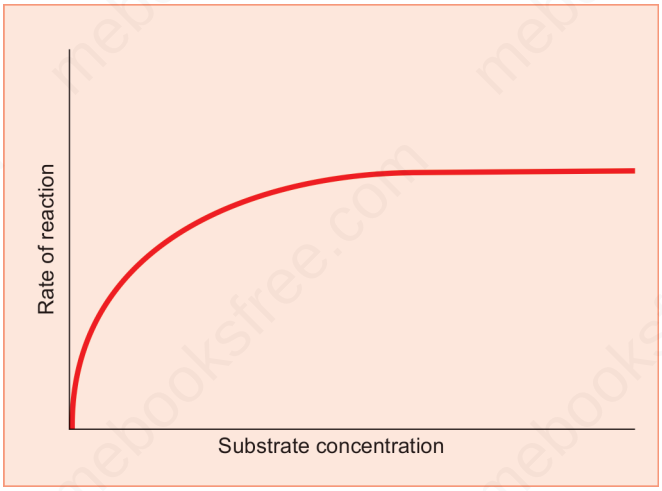


Fig. 3.6: Effect of substrate concentation

Michaelis-Menten Equation

$$Vi = \frac{V_{max} \times S}{Km + S}$$

- Where Vi is the initial velocity
- V_{max} is the maximal velocity
- Km is the Michaelis Constant
- S is the substrate concentration

MICHEALIS CONSTANT (Km)[Q]

- Substrate concentration required to produce half-maximal velocity (½ V_{max})

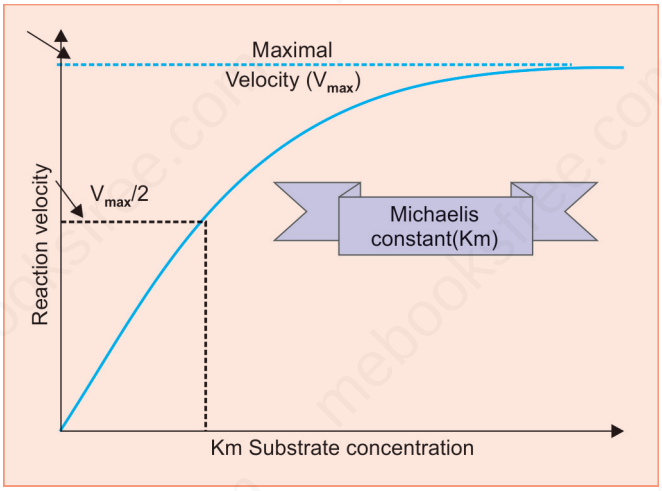


Fig. 3.7: Michaelis constant

Characteristics of Michealis Constant

- Independent of enzyme concentration
- Unique for each enzyme–substrate pair
- Constant for an enzyme—Substrate pair, hence called ***signature of the enzyme***
- Denotes affinity of enzyme to substrate. Lower the Km higher will be the affinity of the substrate
- Km helps to understand the natural substrate of an enzyme
- Substrate with lower Km will be the natural substrate of the enzyme.

Lineweaver Burk Plot

A graphical representation of 1/V on y axis and 1/S on x axis is called Lineweaver Burk Plot or Double Reciprocal plot.

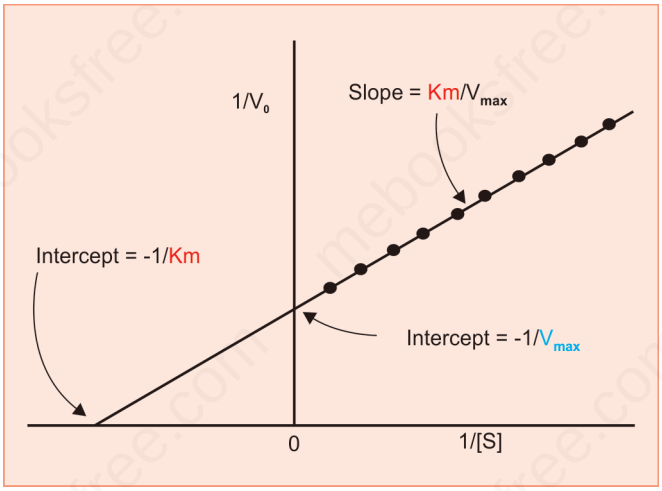


Fig. 3.8: Line weaver burk plot

In Lineweaver burk Plot

- X intercept is –1/Km
- Y intercept is Km/V_{max}
- Slope is Km/V_{max}

Dixon Plot
Alternative to Lineweaver Burk Plot
1/V is measured at different concentration of inhibitor[I], but at same substrate concentration[S].

ENZYME INHIBITION

Types of Enzyme Inhibition

- Competitive inhibition
- Noncompetitive inhibition
- Suicide inhibition
- Allosteric inhibition
- Feedback inhibition.

Competitive Inhibition

A type of inhibition in which the inhibitor compete directly with a normal substrate for an enzyme's substrate binding site.

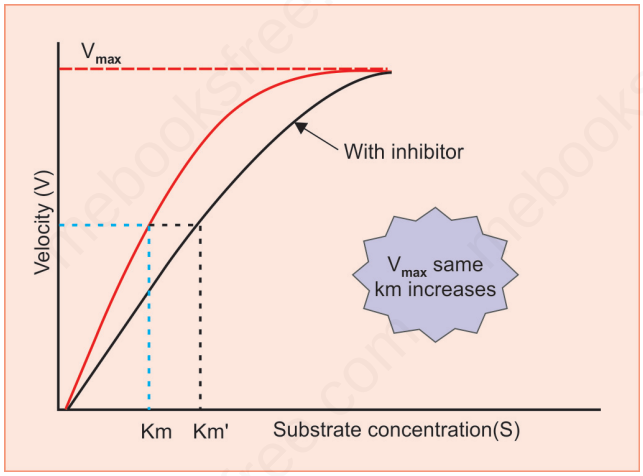


Fig. 3.9: Competitive inhibition

Features of competitive inhibition

- Inhibitor will be structural analogue of substrate
- Reversible
- Excess substrate abolishes inhibition
- V_{max} remains the same
- Km increases.

Examples of Competitive Inhibition

Competitive Inhibitors of enzymes are mostly drugs

Drug	Enzyme inhibited
Statins	HMG CoA Reductase

Contd...

Contd...

Drug	Enzyme inhibited
Dicoumarol	Vitamin K Epoxide
Methotrexate	Dihydrofolate Reductase
Succinyl Choline	Acetyl Choline Esterase

Some competitive inhibitors which are not drugs are

Enzyme	Substrate	Competitive Inhibitor
Lactate Dehydrogenase	Lactate	Oxamate
Aconitase	Cisaconitate	Transaconitate
Succinate Dehydrogenase	Succinate	Malonate
HMG CoA Reductase	HMG CoA	HMG

Noncompetitive Inhibition[Q]

A type of inhibition in which the inhibitor bind to a site distinct from the substrate binding site. Two different types are:

1. Reversible Noncompetitive Inhibition (Only few Noncompetitive inhibition are reversible)
2. Irreversible Noncompetitive Inhibition (Most of Noncompetitive are irreversible).

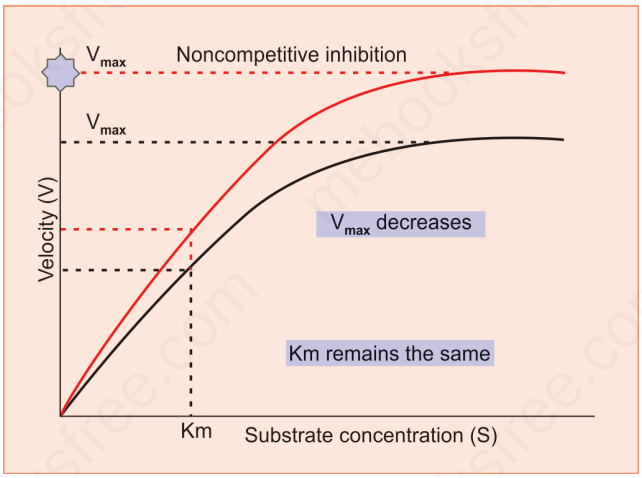


Fig. 3.10: Noncompetitive inhibition

Features of noncompetitive inhibition[Q]

- Inhibitor have no structural resemblance to substrate
- Mostly Irreversible (Except a few reversible non-competitive inhibition)
- Excess substrate do not abolish the inhibition
- Km remains the same
- V_{max} decreases.

Examples of Irreversible Noncompetitive Inhibition

- Are mostly poisonous agents

Noncompetitive Inhibitor	Enzyme
Cyanide[QNBE pattern]	Cytochrome C Oxidase
Iodoacetate	Glyceraldehyde 3 Phosphate
Fluoride	Enolase
Disulfiram(Antabuse)	Aldehyde Dehydrogenase
British Anti-Lewisite (Dimercaprol)	-SH group of several enzymes
Arsenite	Alpha Ketoglutarate Dehydrogenase
Fluoroacetate	Aconitase
Di Isopropyl Flourophosphate	Serine Proteases

Remember
Almost all inhibitors of Electron Transport Chain are examples of irreversible Noncompetitive inhibition.

Suicide Inhibition

- Otherwise called mechanism based inactivation
- Special class of irreversible inhibition
- Inhibitors are relatively unreactive, until they bind with the active site of specific enzyme
- Once the inhibitor binds to the enzyme, by the action of the enzyme, it is converted to a potent inhibitor
- Irreversibly bind to the enzyme and inhibit the enzyme.

Examples of Suicide Inhibition

- Allopurinol inhibit Xanthine Oxidase
  - Allopurinol converted to alloxanthine which irreversibly inhibit the enzyme.
- Treatment of Trypanosomiasis by Difluoromethyl ornithine (DFMO) inhibit Ornithine Decarboxylase
- Aspirin acetylates the active site of Cyclooxygenase, there by inhibiting prostaglandin synthesis.

Feed Back Inhibition

- Also called end-product inhibition
- The activity of the enzyme is inhibited by the final product of the pathway, e.g. AMP inhibits first step in purine synthesis.

REGULATION OF ENZYMES

Regulation of Enzyme Quality (Intrinsic Catalytic Efficiency):

- Allosteric Regulation
- Covalent modification.

Regulation of Enzyme Quantity:

- Control of Enzyme Synthesis (By Induction and Repression)
- Control of Enzyme Degradation.

Allosteric Regulation

Allosteric Enzymes have a catalytic site where the substrate binds and a separate site where a modifier binds.

- If the modifier is an inhibitor it is called allosteric inhibition
- If the modifier is an activator it is called allosteric activation.

Features of allosteric regulation

- The modifier need not be a structural analogue of the substrate
- Partially reversible when excess substrate is added
- Most allosteric enzymes have a quaternary structure and made up of subunits
- Allosteric enzymes occupy key regulatory positions in metabolic pathways, called key enzymes or rate limiting enzymes
- Hill's Equation describes the behavior of enzymes that exhibit cooperative binding of the substrate
- Sigmoid shape of the curve is due to Cooperative Binding.

Cooperative BindingQ
Binding of substrate to one site increases the affinity of binding of the substrate to substrate binding site in other subunits.

Two types of allosteric enzymes

1. *K Series:* In K series allosteric enzyme, Km is raised but V_{max} remains the same.
2. *V series:* In V series allosteric enzyme, V_{max} decreases and Km remains the same.

Comparison of Allosteric regulation and Noncompetitive Inhibition Similarities

- Modifier/Inhibitor is not a structural analogue of the substrate
- Modifier/inhibitor binds to a site distinct from substrate binding site
- V series of Allosteric Enzymes and Noncompetitive inhibition, the Km remains the same and V_{max} decreases.

Differences

Noncompetitive Inbhibition	Allosteric Inhibition
Follow Michaelis-Menten Kinetics	Does not follow Michaelis-Menten Kinetics
Effect of Substrate concentration give a hyperbolic curve	Effect of Substrate concentration give a sigmoid curve

*Examples of Allosteric Enzymes*Q

Enzyme	Activator	Inhibitor
ALA Synthase		Heme

Contd...

Enzyme	Activator	Inhibitor
Aspartate Transcarbamoylase	ATP	CTP
HMG CoA Reductase		Cholesterol
Phosphofructokinase	Fructose 2,6 Bisphosphate	Citrate
Pyruvate Carboxylase	Acetyl CoA	ADP
Acetyl CoA Carboxylase	Citrate	Acyl CoA
Citrate Synthase		ATP
Carbamoyl Phosphate Synthetase I	N Acetyl Glutamate (NAG)	
Carbamoyl Phosphate Synthetase II	PRPP	UTP

Covalent Modification

- Method of regulation of Enzyme activity
- Addition or removal of a group by making or breaking a covalent bond
- By covalent modification enzyme activity is either increased or decreased.

Two types of covalent modification

1. *Irreversible:* Partial proteolysis/zymogen activationQ
2. *Reversible:* Addition/removal of a particular group.

Common methods of reversible covalent modificationQ

- Phosphorylation/dephosphorylation [Most Common covalent modification]
- Methylation
- Adenylation
- ADP ribosylation
- Acetylation.

Examples of covalent modifications

Usually enzymes are in phosphorylated state when body is fasting under the influence of Glucagon. The enzymes that are active in phosphorylated state are

- Glycogen Phosphorylase
- Key enzymes of Gluconeogenesis
- Citrate Lyase
- Phosphorylase Kinase
- HMG CoA Reductase Kinase.

Usually enzymes are in dephosphorylated state when the body is in well fed state under the influence of Insulin.

The enzymes that are active in dephosphorylated state are

- Glycogen synthase
- Key enzymes of Glycolysis
- Acetyl CoA carboxylase
- Pyruvate dehydrogenase
- HMG CoA reductase.

Protein Acetylation: A Ubiquitous Covalent Modification of Metabolic Enzymes

- In recent years, it is found that thousands of other mammalian proteins are subject to modification by covalent acetylation, including nearly every enzyme present in key metabolic pathways
- Acetylation-deacetylation, on the other hand, targets multiple proteins in a pathway, unlike other covalent modifications.
- It has been hypothesized that the degree of acetylation of metabolic enzymes is modulated to a large degree by the energy status of the cell.
- In a well-nourished cell, high level of Acetyl CoA would promote lysine acetylation.
- When nutrients are lacking, acetyl CoA levels drop and the ratio of NAD^+/NADH rises, favoring protein deacetylation.

Acetylation

- Lysine acetyltransferases catalyze the transfer of the acetyl group of acetyl-CoA to the ε-amino groups of lysyl residues, forming N-acetyl lysine.
- In addition, some proteins, particularly those in the mitochondria, become acetylated by reacting
- With acetyl-CoA directly, i.e. without the intervention of an enzyme catalyst.

Deacetylation

Two classes of protein deacetylases have been identified:

- **Histone deacetylases and sirtuins.**
- Histone deacetylases catalyze the removal by hydrolysis of acetyl groups, regenerating the unmodified form of the protein and acetate as products.
- Sirtuins, on the other hand, use NAD^+ as substrate, which yields O-acetyl ADP-ribose and nicotinamide as products in addition to the unmodified protein.

ISOENZYMES

- Physically distinct forms of the same enzymes, but catalyse the same reaction
- Different molecular forms of the same enzyme synthesized from same/various tissues.

Features of Isoenzymes

- They catalyse the same chemical reactions. They differ in heat stability.
  e.g.: heat stable ALP and heat labile ALP.
- They differ in electrophoretic mobility.
  e.g.: CK-I moves faster than CK-3
- They differ in the susceptibility to an inhibitor.
  e.g.: tartarate labile Acid Phosphatase and tartarate stable Acid Phosphatase.
- They differ in subunits they are made up of.
  e.g.: LDH-1 (H4), LDH 5 (M4)
- They differ in tissue localisation.
  e.g.: LDH-1 located in the heart and LDH-5 located in the Muscle.
- They differ in Km value.
  e.g.: Glucokinase (an isoenzyme of Hexokinase) has high Km and but Hexokinase has low Km.

Functional Enzymes and Nonfunctional Enzymes[Q]

Functional Enzymes[Q]

Enzymes which have specific function in the plasma.

Examples of functional enzymes

- Coagulation Factors
- Lipoprotein Lipase.

Nonfunctional Enzymes

- No specific function in the plasma
- Comes out from the tissue as a result of normal wear and tear
- Their level is very low in the serum
- But during tissue injury their level rises in the serum
- Hence they help to diagnose the site of tissue injury.

Examples of nonfunctional enzymes

- LDH, Creatine Kinase, Alkaline Phosphatase
- Does not follow Michaelis–Menten hyperbolic kinetics, instead gives sigmoid Curve.

Isoenzymes of Lactate Dehydrogenase (LDH)

There are five isoenzymes for LDH. It is a tetramer made up of two types of subunits, H and M.

Name of the isoenzyme	Subunit	Tissue localization
LDH-1	H4	Heart Muscle
LDH-2	H3M1	RBC
LDH-3	H2M2	Brain
LDH-4	HM3	Liver and Skeletal Muscle
LDH-5	M4	Liver and Skeletal Muscle

But LDH-5 predominates in Liver

LDHx
A sixth atypical LDH found in male genital tissues.

Isoenzymes of Creatine Kinase (CK)

There are three isoenzymes for Creatine Kinase, made up of two types of subunits, M and B.

Name of the isoenzyme	Subunit	Tissue localization
CK-1	BB	Brain
CK-2	MB	Heart
CK-3	MM	Skeletal Muscle

Two atypical Creatine Kinases are

1. *CK Macro (Macro–CK):* Formed by aggregation of CK-BB with immunoglobulins like IgG.
2. *CK-Mi (Mitochondrial CK):* Found in the exterior surface of Inner mitochondrial membrane of muscle, live and brain.

Isoenzymes of Alkaline Phosphatase (ALP)

Isoenzyme forms of ALP	Tissue of origin
Alpha-1 ALP	Synthesized by epithelial cells of Biliary Canaliculi
Alpha-2 Heat labile ALP	Produced by Hepatic Cells
Alpha-2 Heat stable ALP	Produced by Placenta Inhibited by Phenylalanine
Pre beta ALP	Produced by Osteoblast
Gamma ALP	Produced by Intestinal Cells
Leukocyte ALP	By leukocytes

Regan Isoenzyme
- Named after the first patient from which the enzyme isolated
- An isoenzyme of ALP closely resemble alpha-2 heat stable ALP
- Otherwise called Carcino Placental Isoenzyme
- Elevated in Carcinoma of Lung, Liver, intestine.

Nagao Isoenzyme
- A variant of Regan isoenzyme
- Inhibited by L-leucine.

Cardiac Biomarkers

- Creatine Kinase [CKMB]
- Cardiac Troponin I [CTnI]
- Cardiac Troponin T [CTnT]
- *Brain Natriuretic Peptide [BNP]:* Marker of cardiac failure not a marker of Myocardial Infarction
- Myoglobin
- *Lactate Dehydrogenase [LDH]:* Not used nowadays
- *Aspartate amino Transferase [AST]:* Not used nowadays.

Flipped Pattern of LDH[Q]

Normally LDH-2 is present in higher concentration than LDH-1. But this pattern is reversed in MI. This limited diagnostic importance.

New Cardiac Biomarkers

- Ischemia Modified Albumin
- Glycogen Phosphorylase BB Isoenzyme.
- Pregnancy Associated Plasma Protein A (PAPP-A)
- Myeloperoxidase (MPO).

ENZYME PROFILE FOR LIVER DISEASES

- Enzymes whose elevation in serum reflects damage to hepatocyte
- Aminotransferases (transaminases) are sensitive indicators of liver cell injury
- Are most helpful in recognizing acute hepatocellular diseases such as hepatitis
  - Aspartate aminotransferase (AST) (is found in liver, cardiac muscle, skeletal muscle, kidney, brain, pancreas, lungs, leukocytes, and erythrocytes)
  - Alanine aminotransferase (ALT) (is found primarily in the liver and is therefore a more specific for Liver Disease than AST)
- Enzymes whose elevation in serum reflects cholestasis
  - Alkaline phosphatise (ALP)
  - 5′-nucleotidase
  - γ glutamyltransferase (Transpeptidase) (GGT).

Remember

GGT
- GGT elevation in serum is less specific for cholestasis than are elevations of alkaline phosphatase or 5'-nucleotidase
- GGT is used to identify occult alcohol use.

ALP
- Less than three fold elevation in ALP can be seen in any type of liver disease
- More than four fold elevation of ALP is seen in cholestasis
- ALP elevation is not helpful in distinguishing between intrahepatic and extrahepatic cholestasis.

5' Nucleotidase

Specific for cholestasis than ALP and GGT.

Nonpathologic elevations of ALP seen in:

- Patients over age 60 years
- Type O and Type B blood group
- After eating a fatty meal (due to efflux of gamma ALP)
- In children due to rapid bone growth
- Late normal pregnancy.

AST/ALT Ratio.[Q]

AST:ALT ratio < 1

Any condition causing hepatocellular damage ratio <1 is seen as ALT level rises above AST level. This is because ALT is more specific for hepatocellular damage than AST.
- Chronic viral hepatitis
- Nonalcoholic fatty liver disease
- Toxic hepatitis
- Paracetamol toxicity.

AST: ALT ratio >2:1 is suggestive, while a ratio >3:1
- Highly suggestive of alcoholic liver disease.

Remember

Aminotransferases (ALT and AST)

- ALT is more specific for hepatocellular damage than AST
- In hepatocellular disease ALT elevation is slightly higher than or equal to AST, so AST/ALT ratio is less than 1
- If cirrhosis develop the ratio becomes more than 1
- Minimal ALT elevation less than 300 IU/L is nonspecific. Most likely explanation is Fatty liver
- Level > 1000 IU/L in extensive hepatocellular injury
- In alcoholic liver disease, alcohol-induced deficiency of Pyridoxal Phosphate causes reduced level of transaminases (ALT and AST) ALT level is often normal and AST is rarely > 300 IU/L.

ENZYME PROFILE IN PROSTATE CANCER

Tartrate Labile Acid Phosphatase, Prostate specific antigen (PSA) are enzyme markers of Prostate Cancer.

Prostate specific antigen

- Otherwise called Kallikrein related Peptidase 3 (*KLK3*)
- It is a Serine Protease
- Secreted by epithelial cells of Prostate
- This is Prostate specific, but not prostate cancer specific
- Commonly used cut point for Prostate cancer is PSA level > 4 ng/mL
- But actually there is no PSA level below which risk of Prostate cancer is Zero
- So PSA level estimation should be accompanied by Prostate Biopsy.

ENZYME PROFILE PANCREATITIS

They are:

- Amylase
- Lipase

Serum Amylase is not specific for Pancreatic disease as its level is increased in Parotitis also.

A serum lipase level measurement can be instrumental in differentiating a pancreatic or nonpancreatic cause for hyperamylasemia.

Apart from serum Amylase level can be estimated in urine also.

Biomarkers of Acute Kidney Injury

Novel Biomarkers of Acute Kidney Injury[QQQ]

- Kidney Injury Molecule-1 (KIM-1)
- Neutrophil Gelatin associated Lipocalin (NGAL)
- IL-18
- Alanine Amino Peptidase
- Clusterin

Contd...

Contd...

- Alkaline Phosphatase
- α-Glutathione S-Transferase
- γ Glutamyltranspeptidase
- β2Microglobin
- α-1-Macroglobin
- Retinol Binding Protein
- Cystatin C
- Microalbumin
- Osteopontin
- Liver Fatty Acid Binding Protein
- Sodium–Hydrogen Exchanger Isoform
- Exosomal Fetuin.

MARKERS OF BONE DISEASES

Bone Formation

- Serum Bone-specific Alkaline Phosphatase (BAP)
- Serum Osteocalcin
- Serum Propeptide of type I Procollagen.

Bone Resorption

- Urine and serum cross linked N-telopeptide
- Urine and Serum cross linked C-telopeptide
- Urine total free deoxypyridinoline.

SERINE PROTEASES

- Proteolytic Enzymes with Serine at their active site
- Amino Acid triad in the active site of Serine Proteases-Ser, His, Asp.

Examples of Serine Proteases

- Chymotrypsin
- Trypsin
- Elastase
- Thrombin
- Plasmin
- Complements
- Factor X and XI
- Prostate Specific Antigen.

Serine Proteases Differ in Substrate Specificity[Q]

- Trypsin cleave Basic Amino Acid
- Chymotrypsin cleave hydrophobic bulky amino Acid
- Elastase cleave Small neutral amino Acids like Alanine, Glycine.

BI-BI REACTION

- Kinetic behavior for **two-substrate, two-product reactions** termed 'Bi-Bi' reactions
- Most Bi-Bi reactions follow Michaelis-Menten kinetics.

Bi-bi reactions can be divided into

- An ordered Bi-Bi reaction
  For example: NAD (P) H-dependent oxidoreductases
- A random Bi-Bi reaction
  For example: Many kinases and some dehydrogenases.
- A ping-pong reaction
  For example: Aminotransferases and Serine proteases.[Q]

ENZYMES AS MARKERS OF ORGANELLE AND MEMBRANES[QQQ]

Membrane/Organelle	Marker enzymes
Plasma Membrane	5'- Nucleotidase Adenylyl Cyclase Na^+-K^+ ATPase
Endoplasmic reticulum	Glucose-6-phosphatase
Golgi Complex	Galactosyl Transferase[Q]
Inner Mitochondrial Membrane	ATP Synthase
Peroxisome	Catalase, Urate Oxidase

Contd...

Membrane/Organelle	Marker enzymes
Lysosomes	Cathepsin
Cytoplasm	Lactate Dehydrogenase

ENZYMES AS DIAGNOSTIC REAGENTS

Enzyme	Diagnostic test done
Urease	Urea Estimation
Uricase	Uric Acid Estimation
Glucose Oxidase	Glucose
Hexokinase	Glucose
Peroxidase[Q]	Glucose, Cholesterol
Cholesterol Oxidase	Cholesterol
Creatininase	Creatinine
Lipase	Triglycerides

ENZYMES IN OTHER BODY FLUIDS[Q]

Enzyme	Clinical use
Lactate dehydrogenase in csf, pleural fluid, ascitic fluid	Suggestive of malignant tumor but not confirmatory
Adenosine deaminase in pleural fluid	Suggestive of tuberculous pleural effusion
Amylase in urine	Suggestive of pancreatitis

REVIEW QUESTIONS

GENERAL ENZYMOLOGY

Classification of Enzymes, Enzyme Kinetics

1. Suicidal Enzyme is: **(AIIMS May 2013)**

a. Lipoxygenase
b. Cyclooxygenase
c. Thromboxane Synthase
d. 5′ Nucleotidase

Ans. b. Cyclooxygenase. *(Ref: Harper 30/e p241)*

Cyclooxygenase is a 'Suicide Enzyme'

'Switching off' of prostaglandin activity is partly achieved by a remarkable property of cyclooxygenase—that of self-catalyzed destruction; i.e. it is a 'suicide enzyme.'

Quick review-cyclooxygenase

- Cyclooxygenase (COX) (also called prostaglandin H synthase)
- Involved in prostanoid (prostaglandin. Thromboxane and Prostacyclin) synthesis
- This enzyme has two activities, a cyclooxygenase and peroxidase
- COX is present as two isoenzymes, COX-1 and COX-2.

Drugs acting as inhibitors COX

- NSAID
  - *Aspirin:* Inhibits COX-1 and COX-2.
  - Indomethacin and ibuprofen-inhibit cyclooxygenases by competing with arachidonate.
  - *Coxibs:* Selectively inhibit COX-2
  - Some coxibs have been withdrawn or suspended from the market due to undesirable side effects and safety issues.

- Anti-inflammatory Corticosteroids
  - Transcription of COX-2—but not of COX-1—is completely inhibited by corticosteroids.

2. Which of the following is a Lyase?(JIPMER 2014)

a. Aldolase B
b. Acetyl Co ASynthetase
c. Fatty Acyl CoA Dehydrogenase
d. Acetyl CoA Carboxylase

Ans. a. Aldolase B.

Some examples of Lyases are:

- HMG CoA Lyase
- Argininosuccinate Lyase
- ATP Citrate Lyase
- Aldolase
- Fumarase.

3. All are true about oxygenases, except: (AIIMS Nov 2011)

a. Can incorporate 2 atoms of O_2 in a substance
b. Can incorporate 1 atom of O_2 in a substance
c. Important in hydroxylation of steroids
d. Catalyse carboxylation of drugs

Ans. d. Catalyse carboxylation of drugs.

(Ref: Harper 30/e p118, 119)

- Oxygenases can be monooxygenase or dioxygenase
- Monooxygenase incorporate 1 atom of Oxygen molecule to the substrate
- Dioxygenase incorporate both the atoms of Oxygen molecule to the substrate
- Phase 1 Xenobiotic reactions, **hydroxylation**, catalyzed mainly by members of a class of enzymes referred to as monooxygenases or cytochrome P450s.

4. All of the following enzymes are involved in oxidation-reduction, except: (AI 2009)

a. Dehydrogenases
b. Hydrolases
c. Oxygenases
d. Peroxidases

Ans. b. Hydrolases. *(Ref: Harper 30/e p118, 119)*

Oxidoreductases

Can be:

- Dehydrogenase
- Oxygenase
- Monooxygenase
- Dioxygenase
- Oxidase
- Catalase
- Peroxidase.

Dehydrogenase

Catalyse removal of hydrogen from the substrate. They cannot use oxygen as a hydrogen acceptor.

Oxidases

Removal of hydrogen from a substrate with oxygen as the acceptor of hydrogen.

- Monoamine Oxidase
- Cytochrome Oxidase
- Xanthine Oxidase.

Oxygenases

Catalyze the direct transfer and incorporation of oxygen into a substrate molecule.

Monooxygenases [Mixed Function Oxidases or Hydroxylase]

Incorporate one atom of molecular oxygen into the substrate:

- Phenylalanine Hydroxylases
- 7 alpha Hydroxylases
- Cytochrome p450.

Dioxygenases

Incorporate both atoms of molecular oxygen into the substrate. The basic reaction is shown below:

$A + O_2 \rightarrow AO_2$

Examples of Dioxygenase

- Homogentisate oxidase
- Tryptophan Pyrrolase (Dioxygenase)
- Nitric Oxide Synthase.

5. Enzyme which cleave C-C bond:

a. Lyase
b. Oxidoreductase
c. Ligase
d. Isomerase

Ans. a. Lyase. *(Ref: Harper 30/e p61)*

6. Velocity at Km is:

a. Half the substrate concentration
b. Same as V max
c. Quarter the V max
d. Half the V max

Ans. d. Half the V max.

7. Coenzyme in decarboxylation reaction:

a. Niacin
b. Biotin
c. Pyridoxine
d. Riboflavin

Ans. c. Pyridoxine. *(Ref: Harper 30/e p39, 40)*

- Coenzyme for carboxylation reaction—Biotin
- Coenzyme for decarboxylation—Pyridoxine
- Coenzyme for decarboxylation reactions of Alpha Ketoglutate dehydrogenase and branched chain Ketoacid dehydrogenase—Thiamine.

Enzyme Inhibition

8. The type of enzyme inhibition in which Succinate dehydrogenase reaction is inhibited by malonate is an example of: (AIIMS May 2006)

a. Noncompetitive
b. Uncompetitive
c. Competitive
d. Allosteric

Ans. c. Competitive. *(Ref: Harper 30/e p78)*

Examples of competitive inhibition

Competitive inhibitors of enzymes are mostly drugs

Drug	Enzyme inhibited
Statins	HMG CoA Reductase
Dicoumarol	Vitamin K Epoxide.
Methotrexate	Dihydrofolate Reductase
Succinyl Choline	Acetyl Choline Esterase

Some competitive inhibitors which are not drugs are

Enzyme	Substrate	Competitive inhibitor
Lactate Dehydrogenase	Lactate	Oxamate
Aconitase	Cisaconitate	Transaconitate
Succinate Dehydrogenase	Succinate	Malonate
HMG CoA Reductase	HMG CoA	HMG

9. Features of competitive inhibition is/are: (PGI May 2014)

a. V_{max} increases
b. Km increases
c. V_{max} decreases
d. Km decreases
e. V_{max} constant

Ans. b. Km increases, **e.** V_{max} constant.

Features of competitive inhibition are:

- V_{max} remains a constant
- Km increases
- Reversible.

10. Which is true about enzyme kinetics for competitive inhibition: (JIPMER 2014)

a. Low km high affinity
b. High km high affinity
c. High Km low affinity
d. Low Km low affinity

Ans. c. High Km and lower affinity.

Significance of Km (Michaelis Constant):

- Km is substrate concentration at 1/2 Vmax
- Constant for an enzyme substrate pair
- It is called signature of the enzyme
- Higher the Km, lower is the affinity of the enzyme towards the substrate
- Lower the Km, higher is the affinity of the enzyme towards the substrate.

Features of competitive inhibition:

- Km increases, hence the affinity is lowered
- V_{max} remains the same.

Features of noncompetitive inhibition:

- Km remains the same
- V_{max} decreases.

11. Noncompetitive enzyme inhibition leads to:

a. V_{max} ↑
b. V_{max} ↓
c. V_{max} unchanged
d. Km ↑
e. Km ↓

Ans. b. V_{max} ↓. *(Ref: Harper 30/e p81)*

Features of Noncompetitive Inhibition

- Inhibitor have no structural resemblance to substrate
- Irreversible
- Excess substrate do not abolish the inhibition
- Km remains the same
- V_{max} decreases.

12. True about competitive inhibition of enzyme: (PGI May 2010)

a. ↑ Km
b. ↓ Km
c. ↑ V_{max}
d. No change in Km and Vmax
e. V_{max} remain same

Ans. a. ↑ Km, **e.** V_{max} remain same. *(Ref: Harpers 30/e p81)*

13. Noncompetitive reversible inhibitors:

a. Raise Km
b. Lower Km
c. Lower V_{max}
d. Raise both V_{max} and Km.
e. Do not affect either V_{max} or Km

Ans. c. Lower V_{max}. *(Ref: Harper 30/e p79)*

14. True about competitive antagonism: **(PGI June 2009)**
a. V_{max} increased
b. Substrate analogue
c. Reversible
d. Km increased
e. V_{max} decreased

Ans. b, c, d. *(Ref: Harper 30/e p78-79)*

15. Km changes and V_{max} remains the same. What is the type of Enzyme inhibition?
a. Competitive Inhibition
b. Noncompetitive Inhibition
c. Uncompetitive inhibition
d. Suicide Inhibition

Ans. a. Competitive Inhibition. *(Ref: Harper 30/e p80)*

- Competitive inhibition—Km increases and V_{max} remains the same
- Noncompetitive inhibition—Km remains the same and V_{max} decreases
- Uncompetitive inhibition—Both Km and V_{max} decreases.

Enzyme Regulation

16. Which enzymes catalytic activity is by dephosphorylation **(PGI November 2009)**
a. HMG CoA reductase
b. Glycogen Phosphorylase
c. Citrate Lyase
d. Glycogen Synthase

Ans. a. HMG CoA reductase, **d.** Glycogen Synthase.
(Ref: Harper 30/e p91, Table 9.1)

Enzyme	Activity state	
	Low	High
Acetyl-CoA carboxylase	EP	E
Glycogen synthase	EP	E
Pyruvate dehydrogenase	EP	E
HMG-CoA reductase	EP	E
Glycogen phosphorylase	E	EP
Citrate lyase	E	EP
Phosphorylase b kinase	E	EP
HMG-CoA reductase kinase	E	EP

17. All of the covalent modification regulate enzyme kinetics except:
a. Phosphorylation
b. Acetylation
c. ADP Ribosylation
d. Glycosylation

Ans. d. Glycosylation. *(Ref: Harper 29/e p89)*

Reversible covalent modifications
- Phosphorylation Dephosphorylation
- ADP Ribosylation
- Methylation
- Acetylation.

Irreversible Covalent Modification
- Zymogen activation/ Partial Proteolysis.

18. The following affect enzyme activity except:
a. Methylation
b. Acetylation
c. Induction
d. Phosphorylation

Ans. c. Induction. *(Ref: Harper 30/e p89, 90)*

Induction is method of regulation of enzyme quantity.

Regulation of Enzymes

Can be classified as:

Regulation of Enzyme Quality (Intrinsic Catalytic Efficiency)
- Allosteric Regulation
- Covalent modification.

Covalent modification
- Phosphorylation/dephosphorylation [Most Common covalent modification]
- Methylation
- Adenylation
- ADP ribosylation
- Acetylation.

Regulation of enzyme quantity

Control of Enzyme Synthesis

By Induction and Repression

Control of Enzyme Degradation Enzymes are degraded by Ubiquitin—Proteasome pathway.

19. Chymotrypsinogen is a:
a. Zymogen
b. Carboxypeptidase
c. Transaminase
d. Exopeptidase

Ans. a. Zymogen.

Zymogen activation is an example of irreversible covalent modification.

Serine Proteases

20. Chymotrypsin cleaves carbonyl terminal of: **(PGI May 2011)**

a. Phenylalanine
b. Arginine
c. Lysine
d. Tryptophan
e. Tyrosine

Ans. a, d, e. *(Ref: Harper 30/e p64)*

Serine Proteases

- Proteolytic Enzymes with Serine at their active Site
- Amino acid triad in the active site of Serine Proteases: Ser, His, Asp.

Examples of serine proteases

- Chymotrypsin
- Trypsin
- Elastase
- Thrombin
- Plasmin
- Complements
- Factor X and XI.

Serine proteases differ in substrate specificity

- Trypsin cleave Basic Amino Acid, like Arg, Lys
- Chymotrypsin cleave Hydrophobic Bulky Amino Acid like Trp, Tyr, Phe
- Elastase cleave Small neutral AminoAcids like Alanine, Glycine.

21. Trypsin cleaves: **(PGI May 2010)**

a. Arginine
b. Glutamate
c. Lysine
d. Proline

Ans. a. Arginine, **c.** Lysine. *(Ref: Harper 30/e p63)*

22. A common feature of all serine proteases is: **(AI 2006)**

a. Autocatalytic activation of zymogen precursor
b. Tight binding of pancreatic trypsin inhibitor
c. Cleavage protein on the carboxyl site of serine residues
d. Presence of Ser-His-Asp catalytic triad at the active site

Ans. d. Presence of Ser-His-Asp catalytic triad at the active site. *(Ref: Harper 30/e p62, 63)*

23. Trypsin is a:

a. Serine protease
b. Lecithinase
c. Phospholipase
d. Elastase

Ans. a. Serine Protease. *(Ref: Harper 30/e p63)*

ENZYME AS MARKERS OF ORGANELLE AND MEMBRANE

24. Markers of plasma membrane is/are: **(PGI June 2009)**

a. Galactosyltransferase
b. 5′nucleotidase
c. Adenyl cyclase
d. ATP synthetase
e. Tyrosine

Ans. b. 5′nucleotidase, **c.** Adenylyl cyclase. *(Ref: Harper 30/e, Table 40.2)*

Enzymes as markers of organelle and membranes

Enzymatic markers of different membranes[a]

Membrane	Enzyme
Plasma	5”-Nucleotidase
	Adenylyl cyclase
	Na^+-K^+-ATPase
Endoplasmic reticulum	Glucose-6-Phosphatase
Golgi apparatus	
Cis	GlcNAc transferase I
Medial	Golgi mannosidase II
Trans	Galactosyl transferase
Trans Golgi Network	Sialyl transferase
Inner mitochondrial membrane	ATP synthase

25. Marker enzyme for Golgi apparatus:

a. Galactosyltransferase
b. Glucose 6 Phosphatase
c. 5′ Nucleotidase
d. Catalase

Ans. a. Galactosyltransferase.

Clinical Enzymology

26. True about isoenzymes is: **(AIIMS Nov 2011)**

a. Catalyse the same reaction
b. Same quaternary structure
c. Same distribution in different organs
d. Same enzyme classification with same number and name

Ans. a. Catalyse the same reaction. *(Ref: Harper 30/e p63)*

- Isoenzymes catalyse the same reaction. For example, LDH1-5 all convert Pyruvate to lactate
- They have different quaternary structure. For example, the subunits in LDH-1 is different from LDH-2
- Tissue distribution of each isoform is different
- Enzyme name and number can be different.

27. Nonfunctional enzymes are all except: **(AIIMS Nov 2008)**

a. Alkaline phosphatase
b. Acid phosphatese
c. Lipoprotein lipase
d. Gamma glutamyltranspeptidase

Ans. c. Lipoprotein lipase.

(Ref: Vasudevan and Sreekumari 7/e p301)

FUNCTIONAL ENZYMES AND NONFUNCTIONAL ENZYMES

Functional enzymes

- Enzymes which have specific function in the plasma.

Examples of functional enzymes

- Coagulation Factors
- Lipoprotein Lipase.

Nonfunctional enzymes

- No specific function in the Serum
- Comes out from the tissue as a result of normal wear and tear
- There level is very low in the serum
- But during tissue injury their level rises in the serum
- Hence they help to diagnose the site of tissue injury.

Examples of nonfunctional enzymes are LDH, Creatine Kinase, Alkaline Phosphatase.

28. Peroxidase enzyme is used in estimating: **(AIIMS Nov 2007)**

a. Hemoglobin
b. Ammonia
c. Creatinine
d. Glucose

Ans. d. Glucose.

(Ref: Vasudevan and Sreekumari 7/e p309)

Enzyme	Diagnostic test done
Urease	Urea Estimation
Uricase	Uric Acid Estimation
Glucose Oxidase	Glucose
Hexokinase	Glucose
Peroxidase	Glucose, Cholesterol

Contd...

Contd...

Enzyme	Diagnostic test done
Cholesterol Oxidase	Cholesterol
Creatininase	Creatinine
Lipase	Triglycerides

29. Which of the following estimates blood creatinine level most accurately: **(AIIMS May 2006)**

a. Jaffe method
b. Kinetic Jaffe method
c. Technicon method
d. Enzyme assay

Ans. d. Enzyme Assay

(Ref: Varley's Practical Clinical Biochemistry 6/e p352)

Estimation of blood creatinine

Two Methods:

Chemical Method-Based of Jaffe's Test

In alkaline medium creatinine form a red colored tautomer of Creatinine picrate which is measured colorimetrically. This method can be automated in autoanalysers and Kinetic method can be used.

- Kinetic Jaffe more accurate than Jaffe's Method.

Enzymatic method

- By employing two enzymes Creatininase or Creatinine Deaminase
- More specific
- No interference by Ketones, Bilirubin or Glucose
- Hence measure Creatinine accurately.

30. Not raised in liver disorder is/are: **(PGI may 2013)**

a. Lipase
b. Urease
c. ALP
d. AST
e. ALP

Ans. a. Lipase, **b.** Urease.

(Ref: Harper 29/e p721, 722, Table 48.6)

Test	Aspect of Liver Function Assessed	Major Utility
Serum bilirubin levels (total and conjugated)	Indicator of the ability of the liver to conjugate and excrete bilirubin (conjugation and excretory function)	Aids in the differential diagnosis of jaundice
Total serum protein and albumin	Measure of the biosynthetic function of the liver, as the liver is the primary site of synthesis of most plasma proteins.	Indicator of severity of chronic liver disease

Contd...

Contd...

Test	Aspect of Liver Function Assessed	Major Utility
Prothrombin time	Measure of the biosynthetic function of the liver, as several coagulation factors are synthesized in the liver	Indicator of severity of acute liver disease
Serum enzymes:		
a. Aspartate transaminase (AST)	Serves as marker of injury to hepatocytes that contain AST in abundance	Activities of serum AST and ALT are early indicators of liver damage. They also help in monitoring response to treatment
b. Alanine transaminase (ALT)	Serves as marker of injury to hepatocytes that contain ALT in abundance	
c. Alkaline phosphatase (ALP)	Serves as marker of biliary obstruction	Aids in diagnosis of obstruction of the biliary tract
Blood ammonia	Indicator of the ability of the liver to detoxify ammonia	Levels are elevated in cirrhosis of liver with portal hypertension and in disorders of the urea cycle

31. LDH-5 level elevated in which cell injury:

a. Liver
b. Heart
c. Muscle
d. RBC

Ans. a. Liver. *(Ref: Harper 29/e p66)*

Lactate Dehydrogenase (LDH) is a tetrameric enzyme consisting of two monomer types: H (for heart) and M (for muscle) that combine to yield five LDH isozymes: HHHH (I1), HHHM (I2), HHMM (I3), HMMM (I4), and MMMM (I5). Tissue-specific expression of the H and M genes determines the relative proportions of each subunit in different tissues. Isozyme LDH-I predominates in heart tissue, and isozyme LDH-5 in the liver. Thus, tissue injury releases a characteristic pattern of LDH isozymes that can be separated by electrophoresis.

32. Which of the following LDH is having fastest electrophoretic mobility? (CMC Ludhiana 2014)

a. LDH-1
b. LDH-2
c. LDH-3
d. LDH-5

Ans. a. LDH-1.

- LDH having fastest electrophoretic mobility is LDH-1
- LDH having least electrophoretic mobility is LDH-5
- Creatine Kinase having fastest electrophoretic mobility is CK-1
- Creatine Kinase having least electrophoretic mobility is CK-3.

2

Section | Carbohydrates

CHAPTERS

4. Chemistry of Carbohydrates
5. Metabolism of Carbohydrates

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4 Chemistry of Carbohydrates

Topics Included

- Classification of Carbohydrates
- Glycosaminoglycans
- Mucopolysaccharidoses
- Reactions of Carbohydrates
- Isomerism in Carbohydrates

Carbohydrates are the most abundant organic molecules in nature. The word 'carbohydrate' literally means hydrate of carbon.

DEFINITION

Aldehyde or Keto derivatives of Polyhydric Alcohols or compounds which yield these derivatives on hydrolysis.

General Formula for Carbohydrates[Q] is $C_n(H_2O)_n$, where n = no. of carbon atoms

Main (Primary) source of energy for human beings is Carbohydrates, as Glycogen. (45–65% of total energy)[PGI June 08]

CLASSIFICATION OF CARBOHYDRATES

Carbohydrates are classified into:

- Monosaccharides
- Disaccharides
- Oligosaccharides
- Polysaccharides

Monosaccharides [$C_n(H_2O)_n$]

- Sugars which cannot be further hydrolyzed. They contain one sugar unit
- Building blocks of all carbohydrates.

Depending on the no. of carbon atoms, monosaccharides are subclassified into:

Number of carbon atoms	Generic name
3	Trioses
4	Tetroses
5	Pentoses
6	Hexoses
7	Heptoses
9	Nanoses

Depending on the functional group, monosaccharides are classified into:

- Aldoses with Aldehyde group
- Ketoses with Keto group.

Important monosaccharides based on functional group are:

Generic name	Aldoses	Ketoses
Triose	Glyceraldehyde	Dihydroxyacetone
Tetrose	Erythrose	Erythrulose
Pentose	Ribose Xylose (Epimer of Ribose) Arabinose	Ribulose Xylulose (Epimer of Ribulose]
Hexose	Glucose, Galactose, Mannose	Fructose
Heptose		Sedoheptulose

High-yielding Points—Monosaccharides

- Simplest Carbohydrates of biological interest are: Glyceraldehyde and Dihydroxyacetone.
- Pentoses which constitute a part of Nucleic Acid are Riboses and Deoxyriboses
- Nanoses with biologic significance is Neuraminic Acid.

Sialic Acid

- N-Acyl or O-Acyl derivative of Neuraminic Acid
- N-Acetyl Neuraminic Acid (NANA) is the predominant Sialic Acid
- Constituents of both Glycoprotein and Ganglioside.

RING STRUCTURES OF MONOSACCHARIDES

Monosaccharide molecules of 4, 5 or 6 carbon atoms are quite flexible, and this flexibility brings aldehyde or keto group in close proximity to other hydroxyl groups on the same molecule.

- The reaction of Ketone with hydroxyl group forms Hemiketal Ring Structure
- The reaction of Aldehyde with hydroxyl group forms Hemiacetal Ring Structure.

If the ring structure formed by cyclization is six-sided (made of 5 carbons and 1 oxygen), it is called a Pyranose ring; if it is five-sided (made of 4 carbons and one oxygen), it is called a furanose ring.

- Fructose exists predominantly as Furanose ring structure (Fructofuranose)
- Glucose exists predominantly as Pyranose ring structure (Glucopyranose).

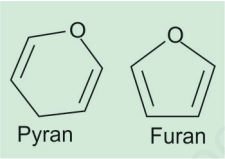


Fig. 4.1: Pyran and furan ring

Biologically Significant Hexoses

Glucose

- Most predominant sugar in human body
- Main source of metabolic fuel of mammals
- Glucose is dextrorotatory, hence otherwise called Dextrose[QNBE model 2012]
- Universal fuel of fetus.

Organs whose Major Energy Source is Glucose

- Brain
- Renal Medulla
- Cornea
- Retina
- Testis
- RBC

Remember

- **The only metabolic fuel for mature erythrocytes in fed state and starving state is Glucose[Q AIIMS May 2015]**

Galactose

- Constituent of Lactose[Q] (Milk sugar)
- Synthesized in the mammary gland for synthesis of lactose
- Part of Glycoprotein, Glycosaminoglycan in Proteoglycans and Glycolipids[Q].

Mannose

- Isolated from plant mannans, hence the name
- Occurs in Glycoproteins and Mucoproteins.

Fructose

- Constituent if Sucrose, the common sugar
- Present in fruit juices, honey[Q] and sugarcane
- Present in the seminal fluid[Q].

Remember

All the Hexoses have a free functional group, hence they are reducing sugars.

DISACCHARIDES [$C_n(H_2O)_{n-1}$]

Two monosaccharide units are linked by a glycosidic bond or yield 2 monosaccharide units on hydrolysis. Depending on their reducing property, they are divided into:

- *Nonreducing disaccharides:* The functional groups are involved in the glycosidic bond formation, hence free functional groups are not available
- *Reducing disaccharides:* Free functional groups are available.

Reducing Disaccharides–Free Functional Group Present [(DNB 1994)]

Disaccharide	Sugar Units[Q]	Linkage
1. Maltose[°]	αDGlucose + αDGlucose	α1→α4 linkage (α1→4 linkage)
2. Isomaltose	αDGlucose + αDGlucose	α1→α6 Linkage (α1→6 linkage)

Contd...

Contd...

Disaccharide	Sugar Units[Q]	Linkage
3. Lactose[°] (Milk Sugar)	DGalactose + β DGlucose	β1→β4 linkage[°]
4. Lactulose	αDGalactose + βDFructose	α1→β4 linkage

Nonreducing Disaccharides[Q]—No Free Functional Group

Disaccharide	Sugar Units	Linkage
Trehalose (Sugar of insect hemolymph, yeast and fungi)	αDGlucose + αDGlucose	α1 → α1 Linkage
Sucrose[Q] (Cane Sugar[Q])	αDGlucose +βDFructose	α1 → β2 Linkage

Lactulose
- Osmotic Laxative
- Mainly Synthetic [small amount in heated milk]
- Not hydrolyzed by intestinal bacteria
- But fermented by intestinal bacteria.

OLIGOSACCHARIDES

Condensation product of 3 to 10 monosaccharides, or yield 3–10 monosaccharide units on hydrolysis:

- Blood group substances are oligosaccharides
- Usually found in association with Proteins (Glycoproteins and Proteoglycans) and lipids (Glycolipids)

POLYSACCHARIDES

Condensation product of more than 10 monosaccharide units or yield > 10 monosaccharide units on hydrolysis. Also called Glycans.

Depending on the type of Monosaccharide units. Polysaccharides are classified into:

- Homoglycans [Homopolysaccharide] Contain only one type of Monosaccharide unit
- Heteropolysaccharides [Heteroglycans] contain different types of Monosaccharide units.

Homoglycans [Homopolysaccharide]

Glycogen

- Storage form of Glucose in animals, hence called animal starch[(Kerala 2006)]
- Made up of αD Glucose
- Branched polymer of Glucose
- α 1,4 Linkage at the linear part and α 1,6 Linkage at branches
- In Muscle Glycogen, granules called β particle are present. These contain 60,000 glucose residues
- In Liver apart from β particle, rosettes of glycogen granules, which are aggregated β particles, are also present.

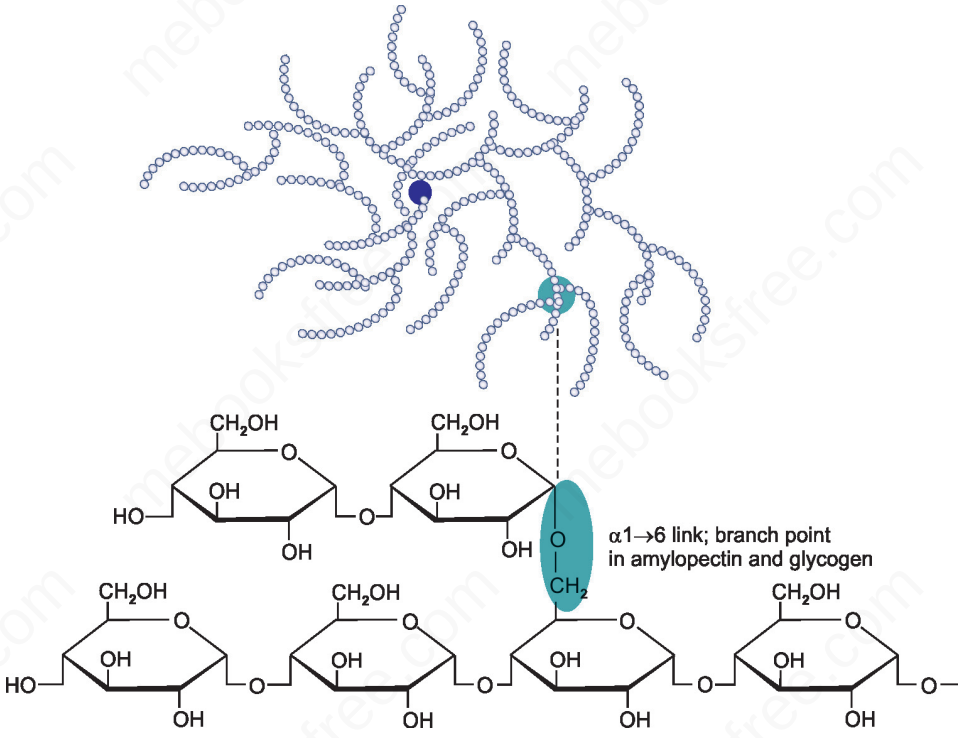


Fig. 4.2: Structure of glycogen

Starch

- Homopolysaccharides made up only of Glucose
- Storage form of Carbohydrates in plants
- Also called glucan or glucosan.

Two main constituents are:

- Amylose (13–20%) which has a nonbranching helical structure
- Amylopectin (80–87%) which consist of branched chains with 24–30 glucose residues.

Starch [Glucosan]		
Mixture of Amylose 15–20% and Amylopectin 80–85%		
(i) Amylose Soluble Unbranched	Glucose	α1→ 4 Linkage
(ii) Amylopectin Insoluble Branched	Glucose	α1→ 4 Linkage α 1→6 Linkage [at Branches]

Chitin

- Found in exoskeleton of crustaceans and insects and in mushrooms
- Made up of N Acetyl D Glucosamine joined by β1 → 4 linkage.

Cellulose

- Chief constituents of plant cell wall
- Homopolysaccharide of βGlucose in β 1, 4 linkage
- Insoluble
- Major component of dietary fiber [Source of bulk in the diet]
- Humans lack enzymes that hydrolyse β 1, 4 glycosidic bonds.Q Hence cannot digest cellulose.

Inulin (Fructosan)

- Homopolysaccharide of Fructose in β 2 → 1 linkage
- Found in roots of dahlias, chicory, onion, garlic, dandelions
- Belongs to a class of fibers
- Readily soluble in water
- Used in clearance test to determine GFR
- Not hydrolyzed by human digestive enzymes.

Dextran

- α Glucose in different linkage (α 1, 6 and α 1, 4 and α 1, 3)
- Used as Plasma Volume Expander.

Plasma Expanders

High mol wt substances that exert colloid osmotic pressure and retain fluid in the vascular component.

Contd...

Contd...

Substances used are:

- Human albumin
- Dextran
- Hydroxyethyl starch (Hetastarch)(AI-94, AI-2002)
- Degraded gelatin polymer.

Compare and Study

Dextrose[Q]	Glucose being dextrorotatory is known as Dextrose in clinical practice
Dextrin	Are intermediates in the hydrolysis of starch
Dextran	Homopolysaccharide made up of Glucose
Lactose	Disaccharide made up of Galactose and Glucose
Lactase	Enzyme which hydrolyze Lactose to Galactose and Glucose
Lactate	Product obtained from Pyruvate during anerobic Glycolysis
Lactulose	Disaccharide made up of Galactose and Fructose

HETEROPOLYSACCHARIDES (HETEROGLYCANS)

- Glycosaminoglycans (Mucopolysaccharides)
- Pectins
- Agarose
- Agar.

Mucopolysaccharides (Glycosaminoglycans[Q])

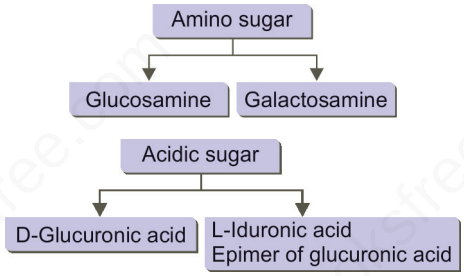
- Glycosaminoglycans are unbranched heteropolysaccharide[Q] chains composed of **Disaccharide repeat units**
- Each disaccharide repeats unit composed of an aminosugar and uronic acid
- They were first isolated from mucin, hence called mucopolysaccharides
- Major component of extracellular matrix.

Properties of GAG

- Carry large number of negative charges [COO^-, Acetyl, Sulphate], these chains tend to repel each other
- Hence slippery consistency of mucous secretion and synovial fluid
- When water squeezes out, they occupy small volume. When compression is released, they spring back to original hydrated volume because of repulsion of negative charges
- Hence, resilient nature of synovial fluid and vitreous humor

- Special ability to bind to large amounts of water. Hence forming major component of extracellular matrix.

Disaccharide repeat unit in glycosaminoglycans (GAG)



Biologically Important GAGs

GAG	Disaccharide Repeat Unit	Location
Hyaluronic Acid (Hyaluronan[Q]	N-Acetyl Glucosamine + Glucuronic Acid[Q]	Skin, Synovial fluid, bone, cartilage, vitreous humor, loose connective tissue, umbilical cord
Chondroitin sulfate	N-Acetyl Galactosamine + Glucuronic Acid	Cartilage, bone, CNS
Keratan sulfate I and II	N-Acetyl Glucosamine, Galactose	**Cornea[Q]** **Cartilage** **Loose connective tissue**
Heparin	Glucosamine, Iduronic Acid	**Mast cells[Q]** **Liver, lung, skin**
Heparan sulfate (HS)	Glucosamine, Glucuronic Acid	Skin Kidney basement membrane
Dermatan sulfate (DS)	N-Acetyl Galactosamine, Iduronic Acid/ Glucuronic Acid	Skin, Wide distribution

Important Points of Glycosaminoglycans

Hyaluronic acid

- Present in bacteria and ECM of nearly all animals
- Play an important role in permitting cell migration during morphogenesis and wound repair.

Chondroitin sulfate

- Join with a protein by the Xylulose-Serine O Glycosidic bond
- Major component of cartilage
- Located at sites of calcification in endochondral bone.

Keratan Sulfate I and II

- Keratan Sulfate I is originally isolated from cornea
- Keratan Sulfate II is isolated from cartilage
- In eye, keratan sulfate lies between the collagen fibrils and play a critical role in corneal transparency.

Heparin

- Consists of Glucosamine and either of two uronic acids
- Vast majority of the uronic acid residues are Iduronic acid
- Initially, all are Glucuronic acid, but 90% of GlcUA is converted to IdUA by a 5′ Epimerase
- Heparin is an anticoagulant
- Heparin specifically binds to Lipoprotein Lipase present in capillary walls, causing release of this enzyme into circulation
- Heparin is found in the granules of mast cells, also liver, lung, and skin.

Heparan sulfate

- Present on many cell surfaces as proteoglycan
- Predominant uronic acid is GlcUA unlike Heparin
- They act as receptors
- Mediate cell growth and cell to cell communication
- Found in kidney basement membrane along with Type IV collagen and laminin
- In kidney basement membrane, it plays a role in charge selectiveness of glomerular filtration.

Dermatan sulfate

- Widely distributed GAG
- The main GAG of skin.

Points to Ponder—GAGs

- GAG with no Uronic Acid[Q]: Keratan Sulfate
- GAG with no Sulfate group: Hyaluronic Acid
- GAG not covalently linked to Protein: Hyaluronic Acid
- GAG found in bacteria: Hyaluronic Acid
- GAG which is an anticoagulant: Heparin
- Most abundant GAG: Chondroitin Sulfate
- Site of Synthesis of GAG: Endoplasmic Reticulum and Golgi
- Shape of Proteoglycan monomer: Bottle Brush
- Glycosaminoglycans are Polyanions
- GAG that helps in cell migration: Hyaluronic acid
- GAGs that have a role in compressibility of cartilage in weight-bearing are Hyaluronic acid and Chondroitin Sulfate
- GAGs that play a role in corneal transparency: Keratan Sulfate I and Dermatan Sulfate
- GAGs that determine charge selectiveness of Renal Glomerular membrane: Heparan Sulfate
- GAGs are generally extracellular
- Some intracellular GAG are Heparin in mast cells and Heparan sulfate in synaptic and other vesicles.

Mucin Clot Test (Rope Test)[Q]

- To detect hyaluronate in the synovial fluid
- Normal synovial fluid forms tight ropy clot on addition of acetic acid.

PROTEOGLYCAN

- GAGs are covalently attached to a protein (termed Core Protein) to form hybrid molecules, called Proteoglycan
- Linking of Polysaccharide chain to core protein occurs by a Core trisaccharide, Gal-Gal-Xyl
- GAG-Core Trisaccharide-Core Protein.

Remember
- **An exception in which GAG not attached to a core protein is Hyaluronic Acid.**

Proteoglycan Monomer and Proteoglycan Aggregate

- Proteoglycan molecules attached to core protein forming proteoglycan monomer
- The shape of Proteoglycan monomers is Bottle brush
- Several Proteoglycan monomer associate noncovalently to a Hyaluronic Acid by a link protein to form Proteoglycan Aggregate.

Proteoglycan	Glycoprotein
>95% Carbohydrate	<5% Carbohydrate
Long linear unbranched Oligosaccharides	Short highly branched Oligosaccharide chains
Disaccharide Repeats	No repeating units

GAG AND DISEASES

Tumor cell migration
- Tumor cells induce fibroblast to synthesize Hyaluronic acid
- Hyaluronic acid permit tumor cells to migrate through ECM
- Some tumor cells have less heparan sulfate at their surfaces, and this may play a role in the **lack of adhesiveness** that these cells display.

GAG and atherosclerosis
- Dermatan Sulfate appears to be the major GAG synthesized by arterial smooth muscle cells
- These cells proliferate in **atherosclerotic lesions** in arteries
- Dermatan sulfate binds plasma low-density lipoproteins
- Because of this, dermatan sulfate may play an important role in development of the atherosclerotic plaque.

GAG and osteoarthritis
- In arthritis, proteoglycans may act as autoantigens
- The amount of chondroitin sulfate in cartilage diminishes with age, whereas the amounts of keratan sulfate and hyaluronic acid increase
- These changes may contribute to the development of osteoarthritis.

MUCOPOLYSACCHARIDOSES (MPS)

Hereditary progressive disease caused by mutation of genes coding for Lysosomal Enzymes needed to degrade GAGs results in Intralysosomal[NBE patternQ2012] accumulation of GAGs.

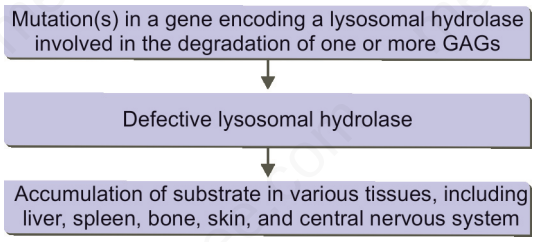


Fig. 4.3: Simplified causation of mucopolysaccharidosis

	Mucopolysaccharidosis	Inheritance	Enzyme Defect	Urinary Metabolite
MPS I H	**Hurler disease[Q]**	**Autosomal Recessive**	**L-Iduronidase[Q]**	Dermatan Sulfate Heparan Sulfate
MPS I S	**Scheie disease**	**Autosomal Recessive**	**L-Iduronidase**	Dermatan Sulfate
MPS II	**Hunter disease[Q]**	**X-linked Recessive**	**Iduronate Sulfatase[Q]**	Dermatan Sulfate Heparan Sulfate
MPS III A	Sanfilippo A disease	Autosomal Recessive	Heparan Sulfate N Sulfatase	Heparan Sulfate
MPS III B	Sanfilippo B disease	Autosomal Recessive	N-Acetyl Glucosaminidase	Heparan Sulfate
MPS III C	Sanfilippo C disease	Autosomal Recessive	Glucosaminide N Acetyl Transferase	Heparan Sulfate
MPS III D	Sanfilippo D disease	Autosomal Recessive	N Acetyl Glucosamine Sulfatase	Heparan Sulfate
MPS IV A	Morquio A	Autosomal Recessive	Galactosamine 6 Sulfatase	Keratan Sulfate Chondroitin 6 Sulfate
MPS IV B	Morquio B	Autosomal Recessive	Beta Galactosidase	Keratan Sulfate
MPS VI	**Maroteaux-Lamy**	Autosomal Recessive	N Acetyl Galactosamine 4 Sulfatase **(Aryl Sulfatase B)**	Dermatan Sulfate
MPS VII	Sly Disease	Autosomal Recessive	Beta - Glucoronidase	Dermatan Sulfate Heparan Sulfate

NB: The important MPS are given in bold letters. Please do learn them.

Recognition Pattern of Mucopolysaccharidosis[Q2013-14]

Clinical features	MPS IH	MPS IS	MPS II	MPS III	MPS IV	MPS VI	MPS VII
Common name	Hurler	Scheie	Hunter	San Filippo	Morquio	Maroteaux-Lamy	Sly Disease
Mental deficiency	+	–	+	+	–	–	?
Coarse facial features	+	(+)	+	–	–	+	?
Corneal clouding	+	+	–	-	(+)	+	?
Visceromegaly	+	(+)	(+)	–	–	+	+
Short stature	+	(+)	+	+	+	+	+
Joint contractures	+	+	+	–	–	+	+
Dysostosis multiplex	+	(+)	(+)	+	+	+	+
Leucocyte inclusions	+	(+)	+	+	–	+	+
Mucopolysaccharidurıa	+	+	+	+	+	+	+

SOME IMPORTANT MUCOPOLYSACCHARIDOSES

Mucopolysaccharidosis-I H (Hurler's Disease)

Biochemical defect

Homozygous or double heterozygous nonsense mutations IDUA gene on Chr 4p encoding α-L-Iduronidase

Clinical features of MPS I H (Hurler's Disease)

- Progressive disorder with multiple organ and tissue involvement that results in premature death, usually by 10 years of age
- An infant with Hurler's syndrome appears normal at birth, but inguinal hernias are often present. Diagnosis is usually made between 6 and 24 mo of age
- **Hepatosplenomegaly, coarse facial features, corneal clouding, large tongue, prominent forehead**, joint stiffness, short stature, and skeletal dysplasia
- Acute cardiomyopathy has been found in some infants < 1 year of age
- Most patients have recurrent upper respiratory tract and ear infections, noisy breathing, and persistent copious nasal discharge
- Valvular heart disease with incompetence, notably of the mitral and aortic valves, regularly develops, as does coronary artery narrowing
- Obstructive airway disease, notably during sleep, may necessitate tracheotomy. Obstructive airway disease, respiratory infection, and cardiac complications are the common causes of death.

Scheie Disease (MPS-IS)

Biochemical defect

A missense mutations in *IDUA* gene on Chr 4p encoding α-L-Iduronidase more likely to preserve some residual enzyme activity associated with a milder form of the disease.

Clinical features

- Similar to MPS IH
- MPS I-S is a comparatively mild disorder characterized by joint stiffness, aortic valve disease, corneal clouding, and mild dysostosis multiplex
- Onset of significant symptoms is usually after the age of 5 years, with diagnosis made between 10 and 20 years of age
- Patients with Scheie disease have **normal intelligence** and **stature** but have significant joint and ocular involvement.

Mucopolysaccharidosis II (Hunter Disease)

Biochemical defect

- X-linked disorder caused by the deficiency of iduronate-2-sulfatase (IDS)
- Point mutations of the *IDS* gene mapped to Xq28 have been detected in about 80% of patients with MPS II
- Hunter disease manifests almost exclusively in males; it has been observed in a few females and this is explained by skewed inactivation of the X chromosome carrying the normal gene.

Clinical features

- MPS II have features similar to those of Hurler disease except for the lack of corneal clouding and the somewhat slower progression of somatic and central nervous system (CNS) deterioration
- Coarse facial features, short stature, dysostosis multiplex, joint stiffness, and mental retardation manifest between 2 and 4 years of age
- Grouped skin papules are present in some patients.

Natowicz syndrome (MPS-IX)

- A genetic defect in hyaluronidase causes MPS IX, a lysosomal storage disorder in which hyaluronic acid accumulates in the joints
- Joint pains and short stature are the clinical features.

Laboratory Diagnosis of Mucopolysaccharidoses

- Urinalysis for presence of increased amounts of GAGs
- Assays of suspected enzymes in white blood cells, fibroblasts, or possibly serum
- Tissue biopsy with subsequent analysis of GAGs by electrophoresis
- Use of specific gene tests
- Prenatal diagnosis can now be performed in at least certain cases using amniotic fluid cells or chorionic villus biopsy.

Points to Ponder—MPS

- Impaired degradation of Heparan Sulfate associated with **Mental Deterioration**
- Impaired degradation of DS, CS, KS associated with **mesenchymal abnormalities**
- All MPS are Autosomal Recessive except Hunter Disease
- Most common MPS is Sanfilippo followed by Hunter and Hurler.

MPS with no Mental Retardation

- Scheie Disease
- Morquio Disease [Keratan Sulphate and Chondroitin Sulphate]
- Maroteaux Lamy Disease

MPS with no corneal clouding

- Hunter's Disease
- Sanfilippo Disease

MPS with no visceromegaly:

- Morquio Disease

Same enzyme deficiency associated with two diseases:

- Hurler Disease
- Scheie Disease

NEWER MODALITIES OF TREATMENT OF MPS

MPS Type	Stem Cell Transplantation (SCT)	Enzyme Replacement	Remarks
I (Hurler, Scheie)	Yes	Aldurazyme	Transplantation before age 2. Enzyme replacement before and after transplantation
II (Hunter Disease)	Questionable	Elaprase	Lack of neurological improvement after SCT
III (Sanfilippo)	No	No	Experimental: Substrate reduction by Flavinoids
IVA (Morquio)	No	Preclinical	Recombinant Galactosamine Sulfatase (GALNS) in course
VI (Maroteaux Lamy Disease)	Yes	Naglazyme	Sustained improvement
VII (Sly)	Questionable	?	Single SCT attempt without neurological improvement

DERIVED SUGARS

Monosaccharides whose structure cannot be represented by general formula or which have some unusual features. They are:

- Acid Sugars (by oxidation of sugars)
- Sugar Alcohols (by reduction of sugars)
- Deoxy Sugars
- Amino Sugars[Q]
- Glycosides
- Furfural Derivative.

Acid Sugars

Formed by oxidation of aldehyde carbon atom, hydroxyl carbon atom or both of monosaccharides.

Under mild oxidation conditions

Aldehyde group is oxidised to produce Aldonic Acid.

- Glucose to Gluconic Acid (TNPGEE 2002)
- Mannose to Mannonic Acid
- Galactose to Galactonic Acid.

Clinical application of glucose to gluconic acid

- During Glucose oxidase method for estimation of blood glucose, Gluconic Acid[Q] is formed from Glucose
- When aldehyde group is protected and last Carbon atom is oxidised, then uronic acid is the product
- Glucose to Glucuronic Acid:
  - Iduronic Acid is an epimer of Glucuronic Acid
  - Constituent of Glycosaminoglycans (GAGs)
  - Used for conjugation of Bilirubin
- Mannose to mannuronic acid galactose to galacturonic acid.

Under strong oxidation condition

- Both first and last carbon atoms are oxidised to produce saccharic acid
  - Glucose to glucosaccharic acid
  - Mannose to mannaric acid
  - Galactose to mucic acid

Clinical application—galactose to mucic acid

Mucic acid forms insoluble crystal forms basis for mucic acid test for the identification of galactose.

SUGAR ALCOHOLS

- Monosaccharides are reduced at their carbonyl group to yield corresponding polyhydroxyalcohols
- Aldoses undergo reduction to form corresponding Alcohol
- Ketoses form two alcohols because of appearance of new asymmetric carbon atom.

Glucose	Sorbitol
Mannose	Mannitol
Galactose	Dulcitol/Galactitol
Fructose	Sorbitol and Mannitol

Clinical Applications of Sugar Alcohols

- Mannitol is used to reduce intracranial pressure by forced diuresis
- Osmotic effect of Dulcitol and Sorbitol causes cataract in Galactosemia and Diabetes respectively
- Polyol pathway is responsible for the development of Diabetic cataract.

DEOXY SUGARS

Hydroxyl group of sugars is replaced by hydrogen atom.

Biochemical Importance of Deoxy Sugars

Deoxyribose

- Oxygen is removed from 2nd position (TNPGEE 04)
- Is an important component of DNA
- Feulgen staining is specific for 2 deoxy sugars (and DNA) in the tissues.

L–Fucose

- Deoxy Sugar present in the Blood group antigens.

2-Deoxy Glucose

- Experimentally an inhibitor of Glucose metabolism.

AMINO SUGARS (HEXOSAMINES)

Aminogroup replaces the hydroxyl group present in the second carbon atom of monosaccharides to form Amino Sugars.

Important amino sugars are:

- Glucosamine
- Galactosamine (Chondrosamine)
- Mannosamine.

Remember

An unusual Amino sugar with 9 carbon atom is Sialic Acid. The principal Sialic Acid found in human body is N Acetyl Neuraminic Acid (NANA)

Points to Ponder—Metabolism of Amino Sugars

- Glucose is the precursor of Amino Sugar.
- The immediate precursor of Glucosamine is Fructose 6 Phosphate[Q 2012].
- Amino group is donated by Glutamine.
- NANA is derived from N Acetyl Mannosamine.

Biochemical significance of amino sugars

- They are components of Glycoproteins, Gangliosides and Glycolipids
- Antibiotic which contains amino sugar is Erythromycin.

GLYCOSIDES

When the monosaccharide is condensed with an alcohol, phenol or sterol by *O-Glycosidic linkage* to form Glycoside. The noncarbohydrate group is called Aglycone.

Clinical Importance of Glycosides

- **Cardiac Glycosides—Action on heart**
  - Digitalis (Steroid is the Aglycone)
  - Quabain.
- **Antibiotics**
  - Streptomycin, Puromycin.

ISOMERISM IN CARBOHYDRATES

Different compounds having same molecular formula are called isomers of one another.

Asymmetric Carbon Atom

The carbon atom to which four different substituent groups are attached is called a chiral or asymmetric carbon atom.

Lebervon't Hoff Rule

The relationship between the number of Asymmetric Carbon atom and the number of Stereoisomers possible.

Number of isomers = 2^n

where n is the number of Asymmetric Carbon atom.

- In Open chain structure of Glucose, there are 4 asymmetric Carbon atoms (C-2, C-3, C-4, C-5)
- But in solution 99.5% Glucose exists in Pyranose form, then first carbon atom also becomes an asymmetric Carbon atom. Hence, the number of isomers possible for Glucose is 2^5 is 32.

Types of Isomers in Carbohydrates

The presence of asymmetric carbon atom imparts two important properties:

- Stereoisomerism
- Opticalisomerism.

Stereoisomerism

Compounds having the same molecular formula but different spatial configuration of H and OH group around the asymmetric carbon atoms.

D and L Isomerism [Enantiomers]

Difference in the orientation of H and OH group around penultimate carbon atom results in two mirror images called D and L isomers (Enantiomers).

The penultimate carbon atom is called Reference Carbon atom.

- The penultimate carbon atom in Glucose and Fructose is C-5
- Most of the naturally occurring Monosaccharides are D isomers (Unlike Amino Acids, which are L isomers).

Examples of Enantiomers[Q]

- D Glucose and L Glucose
- D Fructose and L Fructose
- D Mannose and L Mannose
- D Glyceraldehyde and L Glyceraldehyde.

ANOMERISM

- Formation of ring structure in monosaccharides results in creation of an additional asymmetric carbon called anomeric Carbon atom
- The carbon atom with functional group forms the anomeric carbon atom
- In Glucose C-1 and in fructose C-2 form the anomeric carbon atom
- Difference in orientation of H and OH group around the anomeric carbon atom results in Anomerism
- The resulting isomers are called α and β anomers.

Examples of Anomerism:

- α D Glucose and β D Glucose
- α D Fructose and β D Fructose.

Mutarotation

- Mutarotation is change in optical rotation of plane polarized light with time
- Mutarotation is a property of Anomeric Carbon atom
- Mutarotation is studied by measuring the rotation of plane polarized light
- The optical rotation of α D Glucose is + 112°
- The optical rotation of β D Glucose is + 19°
- Both undergo mutarotation over a period of a few hours, an equilibrium is attained
- At equilibrium the optical rotation is + 52°.

EPIMERISM (DIASTEREOISOMERISM)

Difference in orientation of H and OH group around Carbon atoms other than Anomeric Carbon and Penultimate Carbon results in isomerism referred to as Epimerism.

Epimers of Glucose[Q]

Difference in orientation of H and OH at C2 C3 and C4 for Glucose:

- 2nd Epimer of Glucose – Mannose
- 3rd Epimer of Glucose – Allose
- 4th Epimer of Glucose – Galactose.

OPTICAL ISOMERISM

- When a beam of plane polarized light is passed through a solution of carbohydrates, it rotates the light either to right or to left. Depends on the direction of rotation, two optical isomers possible
- Dextrorotatory (represented by d or +)
- Rotate plane polarized light to right (Clock-wise)
- Levorotatory (represented by l or –)
- Rotate plane polarized light to left (Anticlockwise)
- D Glucose is dextrorotatory (i.e. why glucose is also called Dextrose) but D Fructose is levorotatory.

Racemic Mixture (AI 03)

- ***Equimolar mixture of optical isomers which has no net rotation of plane polarized light.***

Sucrose is Otherwise Called Invert Sugar

- ***Sucrose is dextrorotatory. On hydrolysis of Sucrose yield a mixture of dextrorotatory Glucose and levorotatory Fructose. Because of strong levorotatory nature of fructose, Sucrose on hydrolysis is levorotatory. Hence, Sucrose is called invert sugar***

Points to remember—Isomerism

- Monosaccharide with no Asymmetric Carbon atom-Dihydroxy-acetone
- Ketoses have 1 asymmetric carbon atom less than aldoses

Contd...

Contd...

- No. of Isomers possible is 2^n where n is the no. of Asymmetric carbon atom (Leber von't Hoff rule)
- All D isomers need not be dextrorotatory and vice versa
- Glucose and Fructose are Aldo-Keto Isomers

SHAPES OF OSAZONES

Shape	Sugar
Needle-shaped/Broomstick/ Sheaves of Corn	Glucose, Fructose, Mannose
Pincushion with pins/Hedgehog/ Flower of Touch-me-not	Lactose
Sunflower Petal-shaped	Maltose

NB: Sucrose does not form osazones

TESTS FOR CARBOHYDRATES

General test for all Carbohydrates
- Molisch test

Test for Reducing Substances
- Benedict's Test

Test to differentiate Monosaccharides and Disaccharides
- Barfoed's Test
- Moore's test
- Fehling's Test

Test to differentiate Aldoses and Ketoses
- Seliwanoff's Test
- Rapid furfural Test
- Foulger's Test

Test to detect Deoxy Sugar
- Feulgen Staining

Test for Pentoses
- Bial's Test

Test for Galactose
- Mucic Acid Test.

METHODS FOR ESTIMATION OF GLUCOSE

Reductometric Methods
- Nelson Somogyi Method
- Folin-Wu Method
- O-Toluidine Method

Enzymatic Method
- Hexokinase Method
- Glucose Oxidase Peroxidase Method (GOD–POD)
- Highly Specific Method
- Used in dry analysis technique like Glucometer.

Reaction in glucose oxidase-peroxidase method

Principle of glucose oxidase-peroxidase

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + 4\ \text{aminoantipyrine} + \text{PHBS} \xrightarrow{\text{POD}} \underset{\text{Red color}}{\text{Quinoneimine dye}} + H_2O$$

DIETARY FIBERS[Q]

Complex Carbohydrates not digested by human digestive enzyme. Otherwise called Nonstarch Polysaccharide.

Include:
- **Insoluble Fibers**
  - Cellulose
  - Hemicellulose
  - Lignin
- **Soluble Fibers**
  - Pectin
  - Gums
  - Mucilage

RDA 40 g per 2000 kcal per day is desirable. Supply of energy from dietary fibers is 2 kcal/g.

Beneficial Effects of Dietary Fibers

- Prevent constipation
- *Maintain normal motility of GIT*
- Eliminate bacterial toxin
- Fiber absorbs large quantity of water and toxic compounds by intestinal bacteria
- *Increase bulk of the stool*
- Reduce the stool transit time
- *Decrease GI Cancers—Colon and Rectum*
- *Slow Gastric Emptying*
- Improve Glucose Tolerance by decreasing rate of absorption of glucose
- *Reduce Plasma Cholesterol*
- Decrease absorption of dietary cholesterol[Q]
- Bind the bile salt and decrease enterohepatic circulation of bile salts and increase excretion of bile salt, the excretory form of cholesterol
- Give sensation of stomach fullness.

A High-fiber diet is associated with reduced incidence of[Q]:
- Diverticulosis
- Cancer Colon
- Cardiovascular Disease
- Diabetes Mellitus

Dietary Fiber neither digested nor fermented—Lignin[Q]

Digestion of dietary fibers by colonic bacteria in herbivores give rise to small chain fatty acids (Acetate, Propionate and Butyrate[Q])

AIIMS Nov 2001

DIGESTION OF CARBOHYDRATES

By two sets of enzymes:
1. Alpha Amylases
2. Disaccharidases.

Action of Alpha Amylase (AIIMS Dec 94)

- Cleaves alpha 1,4 glycosidic bond
- Present in saliva, Pancreatic juice and Intestinal juice
- Yielding dextrins, then a mixture of glucose, maltose, and maltotriose and small branched dextrins (from the branchpoints in amylopectin).

Action of Disaccharidases

- They are maltase, sucrase-isomaltase (a bifunctional enzyme catalyzing hydrolysis of sucrose and isomaltose), lactase, and trehalase
- They are located on the brush border of the intestinal mucosal cells
- Yield the corresponding monosaccharides, which are absorbed.

Lactase Deficiency and Sucrase Deficiency[Q]

Lactase Deficiency	Sucrase Deficiency (AI 04)
Clinical manifestation following ingestion of milk, which contain Lactose	Clinical manifestation following ingestion of dairy products which contain Sucrose
Watery Diarhea Bloating Failure to thrive	Watery Diarhea Bloating Failure to thrive

ABSORPTION OF CARBOHYDRATES

By two sets of transporters:
1. Sodium-dependent Glucose transporters (SGLT).
2. Sodium-independent Glucose transporters (GLUTs).

Sodium-dependent Glucose Transporters

- Secondary Active Transport
- Unidirectional
- SGLT is coupled with Na-K+ ATPase pump. Sodium-dependent Glucose Transporters (SGLT).

SGLT1	Small Intestine, Renal Tubules	Absorption of Glucose
SGLT2	Renal Tubules	Absorption of Glucose

Clinical Correlation—Renal Glycosuria
Isolated glucosuria in the presence of a normal blood glucose concentration is due to mutations in SLC5A2, the gene that encodes the high-capacity sodium-glucose co-transporter SGLT2 in the proximal renal tubule

Glucose Transporters

- Passive process down the concentration gradient
- Bidirectional
- Facilitative Diffusion
- Ping Pong Mechanism
- Sodium-independent.

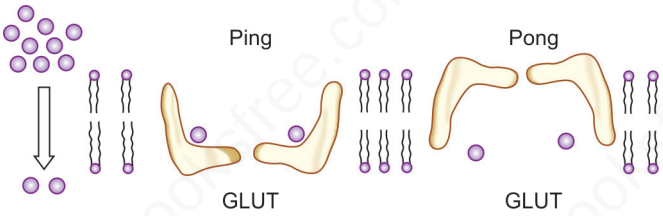


Fig. 4.4: Glucose transporters—Ping pong mechanism

Transporter	Tissue Location	Function
GLUT 1	**Brain**, Kidney, Colon, Placenta, **RBCs**, Retina	Basal Glucose Uptake
GLUT 2	**Liver Sinusoid membrane β cells of Pancreas,** Serosal side (basolateral side) of Intestinal Cell Basolateral membrane of PCT in Kidney	In liver, removal of excess glucose from blood; In pancreas, regulation of insulin release. Low affinity and higher Km.
GLUT 3	**Neurons**, Placenta, Kidney	High affinity for Glucose
GLUT 4	Heart, Skeletal Muscle, Adipose Tissue	**Insulin-dependent** Glucose Uptake
GLUT 5	Small Intestine, Testis, Sperm	Primarily **Fructose Transporter[Q]**
GLUT 6	Spleen, Leukocytes	Possibly no transporter function
GLUT 7	Liver Endoplasmic reticulum	Glucose Transporter in the Endoplasmic Reticulum

Other Glucose transporters are:

Glucose Transporter	Tissues where expressed	Functions
GLUT 8	Testis, **Blastocyst**, brain	**Insulin-responsive** glucose transporter of Blastocyst. Glucose transporter to mature spermatozoa
GLUT 9	Liver, Kidney	**Urate transporter**
GLUT 10	Liver, Pancreas	
GLUT 11	Heart, Skeletal muscle	**Fructose transporter**
GLUT 12	Prostate, Heart, Mammary gland, White adipose tissue	**Insulin responsive**

Inhibitors of Glucose Transporters

- Phlorizin (Phloretin 2 β Glucoside)[Q(PGI Dec 2008)] is an inhibitor of Sodium-dependent glucose transporter by competing with D Glucose-binding sites of the carrier. SGLT2 > SGLT1
- Phloretin (Aglycone of Phlorizin) is an inhibitor of facilitated diffusion by GLUT-1 or GLUT-4.

Points to Ponder—Glucose Transporters

- Widely distributed Glucose transporter-GLUT-1
- Most abundant Glucose transporter in RBC-GLUT-1
- Major glucose transporter of Brain-GLUT-1
- Major glucose transporter of neurons-GLUT-3
- Major glucose transporter of Placenta-GLUT-1
- Glucose transporter of blastocyst-GLUT-8
- Insulin-dependent Glucose transporters-GLUT-4, GLUT-8, GLUT-12
- GLUT-3 is present in neurons, whereas GLUT-1 is not present in neurons
- Urate transporter is GLUT-9

ABSORPTION OF MONOSACCHARIDES

- Glucose and galactose are absorbed by a sodium-dependent process.
- They are carried by the same transport protein (SGLT 1) and compete with each other for intestinal absorption
- Fructose absorbed down their concentration gradient by GLUT 5
- All the sugars exit from intestinal cells via GLUT 2.

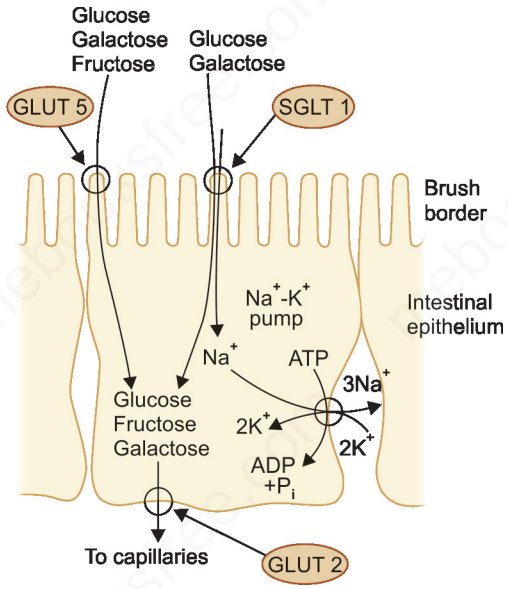


Fig. 4.5: Absorption of monosaccharides

REVIEW QUESTIONS

CLASSIFICATION OF CARBOHYDRATES (INCLUDES TESTS AND REACTIONS OF CARBOHYDRATES)

1. In Benedict's test, red color is/are produced by: **(PGI Nov 2014)**

a. Sucrose
b. Inositol
c. Fructose
d. Lactose
e. Maltose

Ans. c. Fructose, **d.** Lactose, **e.** Maltose.

(Ref: Varleys: Practical Clinical Biochemistry 4/e p110)

Benedict's test is a test for reducing substances in urine.

Carbohydrates that give positive Benedict's test are:

- Pentoses, Fructose, Glucose, Galactose
- Reducing disaccharides like Lactose, Maltose, Isomaltose

Noncarbohydrates that give Benedict's test positive are:

- Homogentisic acid
- Glucuronic acid
- Salicylates
- Ascorbic acid
- Uric acid
- Glutathione
- Creatinine in very high amount

Nonreducing disaccharides, like Sucrose and Trehalose do not give positive Benedict's test. Tests for reducing substances are Benedict's test and Fehling's test.

2. Which of the following is not an aldose? **(PGI 2012)**

a. Glucose
b. Mannose
c. Fructose
d. Galactose
e. Glycerol

Ans. c. Fructose, **e.** Glycerol.

Generic name	Aldose	Ketose
Triose	Glyceraldehyde	Dihydroxy Acetone
Tetrose	Erythrose	Erythrulose
Pentose	Ribose Xylose (Epimer of Ribose) Arabinose	Ribulose Xylulose (Epimer of Ribulose]
Hexose	Glucose, Galactose, Mannose	Fructose
Heptose		Sedoheptulose

3. Glucose detection can be done by all except: (Kerela 2010)

a. Glucose oxidase
b. Ferric Chloride test
c. Dextrostix
d. Folin-Wu method

Ans. c. Ferric Chloride Test.

Ferric Chloride test is the test done in Alkaptonuria and Phenyl Ketonuria.

Methods for estimation of glucose

- Reductometric Methods
- Nelson-Somogyi Method
- Folin-Wu Method
- O-Toluidine Method

Enzymatic method

Hexokinase Method-Glucose Oxidase-Peroxidase Method (GOD –POD) **(AIIMS Nov 2007)**

- Highly Specific Method
- Used in dry analysis technique like Glu-cometer.

Reaction

Principle of Glucose Oxidase Peroxidase

$$\text{Glucose} + H_2O + O_2 \xrightarrow{GOD} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + 4\text{ aminoantipyrine} + PHBS \xrightarrow{POD} \text{Quinoneimine dye} + H_2O$$

Red color

4. Which of the following carbohydrate metabolisms is used for liver function assessment: (Kerela 2010)

a. Galactose tolerance test
b. Sucrose tolerance test
c. Glucose tolerance test
d. Lactose tolerance test

Ans. a. Galactose tolerance test.

(Ref: Vasudevan and Sreekumari 7/e p355)

Galactose Tolerance test assesses the metabolic capacity of liver

Galactose is almost entirely metabolized in the liver.

5. $C_n(H_2O)$ n is the formula for: (Kerela 2009)

a. Monosaccharide
b. Disaccharide
c. Polysaccharide
d. Oligosaccharide

Ans. a. Monosaccharide.

(Ref: Vasudevan and Sreekumari 7/e p69)

6. All are reducing sugars except (Kerala 2009)

a. Sucrose
b. Lactose
c. Glucose
d. Fructose

Ans. a. Sucrose. *(Ref: Vasudevan and Sreekumari 7/e p77)*

All monosaccharides have free functional group and hence they are reducing sugars.

Reducing Disaccharides–Free Functional group present *(DNB 1994)*

Disaccharide	Sugar Units[Q]	Linkage
1. Maltose[Q]	αDGlucose + αD G lucose	α 1 → α4 Linkage (α1 → 4 linkage)
2. Isomaltose	αDGlucose + αD Glucose	α 1 → α6 Linkage (α1 → 6 linkage)
3. Lactose[Q] (Milk Sugar)	DGalactose + βD Glucose	β1 → β 4 Linkage[Q]
4 Lactulose	αDGalactose + βD Fructose	α1 → β4 Linkage

Nonreducing Disaccharides[Q]—No Free Functional group

Disaccharide	Sugar Units	Linkage
Trehalose	Glucose + Glucose	α1β → 1 Linkage
Sucrose[Q]	Glucose + Fructose	α1 → β2 Linkage

Isomerism in Carbohydrates

7. Which of the following are enantiomers: (PGI May 2011)

a. D-Galactose and L-Glucose
b. d- Galactose and I-Glucose
c. D-Mannose and L-Mannose
d. d-Mannose and I-Mannose
e. D-glucose and L-Glucose

Ans. c. D-Mannose and L-Mannose,
e. D-Glucose and L-Glucose.
(Ref: Harper 29/e p133, Harper 30/e p153)

D and L Isomerism [Enantiomers]

Difference in the orientation of H and OH group around Penultimate Carbon atom results in two mirror images called D and L isomers (Enantiomers).

The penultimate carbon atom is called Reference Carbon atom. The penultimate carbon atom in Glucose and Fructose is C-5.

Most of the naturally occurring Monosaccharides are D isomers [Unlike Amino Acids, which are L isomers.]

Examples of EnantiomersQ

D Glucose and L Glucose
D Fructose and L Fructose
D Mannose and L Mannose
D Glyceraldehyde and L Glyceraldehyde.

8. Epimer combination(s) is/are: **(PGI May 2010) (Nov 2009)**

a. D-glucose and D-fructose
b. D-mannose and D-talose
c. D-glucose and D-mannose
d. D-glucose and D-gulose
e. D-galactose and D-glucose

Ans. c. D-glucose and D-mannose,
e. D-galactose and D-glucose. *(Ref: Harper 30/e p153)*

Epimerism [Diastereoisomerism]

Difference in orientation of H and OH group around Carbon atoms other than Anomeric Carbon and Penultimate Carbon results in isomerism refered to as Epimerism.

Epimers of GlucoseQ

Difference in orientation of H and OH at C2 C3 and C4 for Glucose:

- 2nd Epimer of Glucose – Mannose
- 3rd Epimer of Glucose – Allose
- 4th Epimer of Glucose– Galactose.

Glycosaminoglycans and Mucopolysaccharidoses

9. Glycosaminoglycans present in cornea: **(NBE pattern Qn)**

a. Keratan Sulfate
b. Hyaluronic acid
c. Chondroitin Sulfate
d. Dermatan Sulfate

Ans. a. Keratan Sulfate.
(Ref: Harper 30/e p637)

- **KS-I Originally isolated from Cornea**Q
- KS-II from Cartilage
- The composition of both Keratan sulfate are same (N-acetyl Glucosamine and Galactose)
- No Uronic acid in Keratan Sulfate
- In the eye, they lie between collagen fibril and play a critical role in corneal transparency.

10. Which deposition results in cataract? **(NBE pattern Qn)**

a. Glucose
b. Galactose
c. Sugar amines
d. Sugar alcohols

Ans. d. Sugar alcohol. *(Ref: Harper 30/e p205)*

- In Diabetes mellitus, in the lens by polyol pathway Glucose converted to Sorbitol by the enzyme Aldose reductase
- In galactosemia, Dulcitol or Galactictol is responsible for cataract
- Sorbitol, Dulcitol are sugar alcohols.

11. Cellulose is: **(NBE Pattern Q)**

a. Complex Lipoprotein
b. Starch Polysaccharide
c. Nonstarch Polysaccharide
d. Complex Glycoprotein

Ans. c. Nonstarch Polysaccharides.
(Ref: Harper 30/e p153)

Foods contain a wide variety of other polysaccharides that are collectively known as nonstarch polysaccharides; they are not digested by human enzymes, and are the major components of dietary fiber.

Examples are cellulose from plant cell walls (a glucose polymer) and inulin, the storage carbohydrate in some plants (a fructose polymer).

12. A 4-year-old boy with mental retardation, dysostosis multiplex, coarse facial features, clear cornea. What is the diagnosis?

a. MPS Type IV
b. Hunter's Disease
c. Hurler's Disease
d. Zellweger Syndrome

Ans. b. Hunter's Disease.
(Ref: Nelson 20/e Tables 82.1 and 82.2)

Recognition pattern of mucopolysaccharidoses

Clinical features	MPS IH	MPS IS	MPS II
Common name	Hurler	Scheie	Hunter
Mental deficiency	+	–	+
Coarse facial features	+	(+)	+
Corneal clouding	+	+	–
Visceromegaly	+	(+)	(+)
Short stature	+	(+)	+
Joint contractures	+	+	+
Dysostosis multiplex	+	(+)	(+)
Leucocyte inclusions	+	(+)	+
Mucopolysacchariduria	+	+	+

13. Mucopolysaccharide that doesn't contain Uronic acid residue is: (JIPMER 2015)

a. Heparan Sulfate
b. Heparin
c. Chondroitin Sulfate
d. Keratan Sulfate

Ans. d. Keratan Sulfate.

GAG with no Uronic Acid^{o}-Keratan Sulfate
GAG with no Sulfate group-Hyaluronic Acid
GAG not covalently linked to Protein-Hyaluronic Acid.

14. Mucopolysacchridoses, which are a lysosomal storage disease, occur due to abnormality in: (PGI May 2015)

a. Hydrolase enzyme
b. Dehydrogenase enzyme
c. Lipase enzyme
d. Phosphatase
e. Acetyl CoA Carboxylase

Ans. a. Hydrolase enzyme.

(Ref: Nelson 20/e Chapter Defects in the metabolism of lipids/lysosomal storage disorder)

The lysosomal storage diseases are diverse disorders each due to an inherited deficiency of a lysosomal **hydrolase l**eading to the intralysosomal accumulation of the enzyme's particular substrate.

15. Heparin is a: (NBE Pattern Qn)

a. Glycosaminoglycan
b. Polysaccharide
c. Proteoglycan
d. Carbohydrate

Ans. a. GAG. *(Ref: Harper 30/e p637)*

- Heparin is a Glycosaminoglycan
- Glycosaminoglycans are heteropolysaccharides
- Disaccharide repeat unit is Glucosamine and Iduronic acid
- Initially uronic acid present is Glucuronic acid, 5 epimerase convert 90% of GlcUA to IdUA
- Heparin is found in the granules of mast cells, also in lung, liver and skin. Heparin specifically binds to lipoprotein lipase present in capillary walls and cause its release into circulation
- Heparin is an anticoagulant.

16. Glycogenin is a: (NBE pattern Qn)

a. Polypeptide
b. Polysaccharide
c. Lipid
d. Glycosaminoglycan

Ans. a. Polypeptide. *(Ref: Harper 30/e p177)*

- Glycogenin is a protein
- 37 K Da protein
- Glycogenin catalyzes transfer of 7 glucosyl residues from UDPGlucose, in alpha 1 → 4 linkage
- Glucosyl residues are added on specific tyrosine residues of Glycogenin
- Glycogenin remains at the core of Glycogen granule.

17. Complex polysaccharides are converted to glucose and absorbed by the help of: (Kerala 2007)

a. Na^+K^+ ATPase
b. Sucrase
c. Enterokinase
d. Carboxypeptidase

Ans. b. Sucrase. *(Ref: Harper 30/e p538)*

The cells of brush border of intestine contain the enzyme, Sucrase, Maltase, Iso-maltase and lactase hydrolyse corresponding disaccharides to monosaccharides which are absorbed.

Glucose Transporters

18. After overnight fasting, levels of glucose transporters are reduced in: (May 2010)

a. Brain cells
b. RBCs
c. Adipocyte
d. Hepatocyte

Ans. c. Adipocytes. *(Ref: Harper 29/e p158, 30/e p192)*

GLUT-4 and Insulin

Glucose uptake into muscle and adipose tissue is controlled by insulin, which is secreted by the islet cells of the pancreas in response to an increased concentration of glucose in the portal blood.

In the fasting state, the glucose transporter of **muscle and adipose tissue (GLUT-4)** is in intracellular vesicles.

An early response to insulin is the migration of these vesicles to the cell surface, where they fuse with the plasma membrane, exposing active glucose transporters.

These insulin-sensitive tissues only take up glucose from the bloodstream to any significant extent in the presence of the hormone.

As insulin secretion falls in the fasting state, so that the receptors are internalized again, reducing glucose uptake.

19. Glucose transporter in myocyte stimulated by insulin is: (AIIMS Nov 2009)

a. GLUT-1
b. GLUT-2
c. GLUT-3
d. GLUT-4

Ans. d. GLUT-4. *(Ref: Harper 29/e p158, 30/e p191)*

Glucose transporters which are insulin-responsive, are GLUT 4, GLUT 8 and GLUT 12.

- GLUT 4 is present in the Heart, skeletal muscle, adipose tissue
- GLUT 8 is present in the Testis, **blastocyst**
- GLUT 12 is present in Heart, prostate, white adipose tissue, mammary gland.

20. Defect in renal glucosuria: (NBE Pattern Q)

a. GLUT 1
b. GLUT 2
c. SGLT 1
d. SGLT 2

Ans. d. SGLT2. *(Ref: Harrison 19/e p299)*

Renal Glucosuria

Isolated glucosuria in the presence of a normal blood glucose concentration is due to mutations in SLC5A2, the gene that encodes the high-capacity sodium-glucose co-transporter SGLT2 in the proximal renal tubule

21. Regarding glucose transporters [GLUT], incorrect match is:

a. GLUT-5: intestines and kidney
b. GLUT-4: adipose tissue
c. GLUT-3: placenta
d. GLUT-2: beta cell glucose sensor

Ans. None. *(Ref: Harper 29/e p158, 30/e p191)*

Location of glucose transporters

Transporter	Tissue Location
GLUT 1	Brain, Kidney, Colon, Placenta, RBCs, Retina
GLUT 2	Liver, **β cells of Pancreas**, Serosal side of Intestinal Cell Basolateral membrane of PCT in Kidney.
GLUT 3	Neurons, **Placenta,** Kidney
GLUT 4	Heart, Skeletal Muscle, **Adipose Tissue**
GLUT 5	**Small Intestine,** Testis, Sperm **Kidney**
GLUT 6	Spleen, Leukocytes
GLUT 7	Liver Endoplasmic reticulum
GLUT 8	Testis, Blastocyst, Adipose tissue, Brain
GLUT 9	Liver, Kidney
GLUT 12	Heart, Prostate, mammary gland, White adipose tissue

22. Facilitated transport of glucose that is insulin insensitive (non-dependent) takes place in:

a. Skeletal muscle
b. Liver
c. Adipose tissue
d. Heart

Ans. b. Liver. *(Ref: Harper 29/e p158, 30/e p191)*

Insulin responsive Glucose transporters are

- GLUT 4-Heart, Skeletal Muscle, Adipose tissue
- GLUT 8-Testis, Blastocyst, Brain, Adipose tissue
- GLUT 12-Heart, Prostate, White adipose tissue, mammary gland.

23. Glucose transporter present in the RBC: (NBE pattern Qn)

a. GLUT-1
b. GLUT-2
c. GLUT-3
d. GLUT-4

Ans. a. GLUT-1. *(Ref: Harper 29/e p158, 30/e p191)*

- Highest level of GLUT1 is present in the RBC
- Major Glucose transporter in brain is GLUT1 (not present in neurons)
- Major Glucose transporter in the Placenta is GLUT 1
- Major Glucose transporter in the RBC is GLUT1
- Major neuronal Glucose transporter is GLUT3

- Insulin-responsive glucose transporter is GLUT4, GLUT 8 and GLUT 12
- Fructose transporter GLUT 5 (mainly) and GLUT 11
- Urate transporter is GLUT 9
- Glucose transporter in blastocyst is GLUT 8.

Digestion and Absorption of Carbohydates

24. Inulinlike fructosans are used as prebiotics as they are nondigestible. Resistance to digestion in the upper GI tract results from: (AI 2010)

a. Absence of digestive enzyme in the upper GIT
b. Beta configuration of anomeric C2
c. Low pH of the stomach
d. Presence of α-Glycosidic linkage

Ans. b. Beta configuration of anomeric C2.

(Ref: Harper 29/e p137, 30/e p156)

- Inulin is a polysaccharide of fructose (and hence a fructosan)
- *Inulin consists of fructose polymer linked β 2 $\rightarrow$ 1*
- Found in tubers and roots of dahlias, artichokes, and dandelions
- It is readily soluble in water and is used to determine the glomerular filtration rate
- It is not hydrolyzed by intestinal enzymes as mammals lack any enzyme that hydrolyzes the β1 $\rightarrow$ 4 bonds.

25. Complex polysaccharides are converted to glucose and absorbed with the help of: (Kerala 2007)

a. Na^+K^+ ATPase
b. Sucrase
c. Enterokinase
d. Carboxypeptidase

Ans. b. Sucrase. *(Ref: Harper 30/e p538)*

- The cells of brush border of intestine contain the enzyme, Sucrase, Maltase, Iso-maltase and lactase hydrolyse corresponding disaccharides to monosaccharides which are absorbed.

Digestion of Carbohydrates

Amylases Catalyze the Hydrolysis of Starch

The hydrolysis of starch is catalyzed by salivary and pancreatic amylases, which catalyze random hydrolysis of (α1 $\rightarrow$ 4) glycoside bonds, yielding dextrins, then a mixture of glucose, maltose, and maltotriose and small branched dextrins (from the branchpoints in amylopectin).

Disaccharidases are Brush Border Enzymes

The disaccharidases, maltase, sucrase-isomaltase (a bifunctional enzyme catalyzing hydrolysis of sucrose and isomaltose), lactase, and trehalase are located on the brush border of the intestinal mucosal cells. They act on corresponding disaccharides and monosaccharides are formed.

Enzyme	Monosaccharides formed
Maltase	Glucose + Glucose
Sucrase-Isomaltase	Glucose + Fructose Glucose + Glucose
Lactase	Galactose + Glucose
Trehalase	Glucose + Glucose

5 Metabolism of Carbohydrates

Topics Included

- **Major metabolic Pathways**
  - Glycolysis
  - Gluconeogenesis
  - Glycogenesis
  - Glycogenolysis
- **Minor Metabolic Pathways**
  - HMP Shunt Pathway
  - Uronic Acid Pathway
  - Polyol Pathway
  - Fructose Metabolism
  - Galactose Metabolism

GLYCOLYSIS (EMBDEN-MEYERHOF PATHWAY)

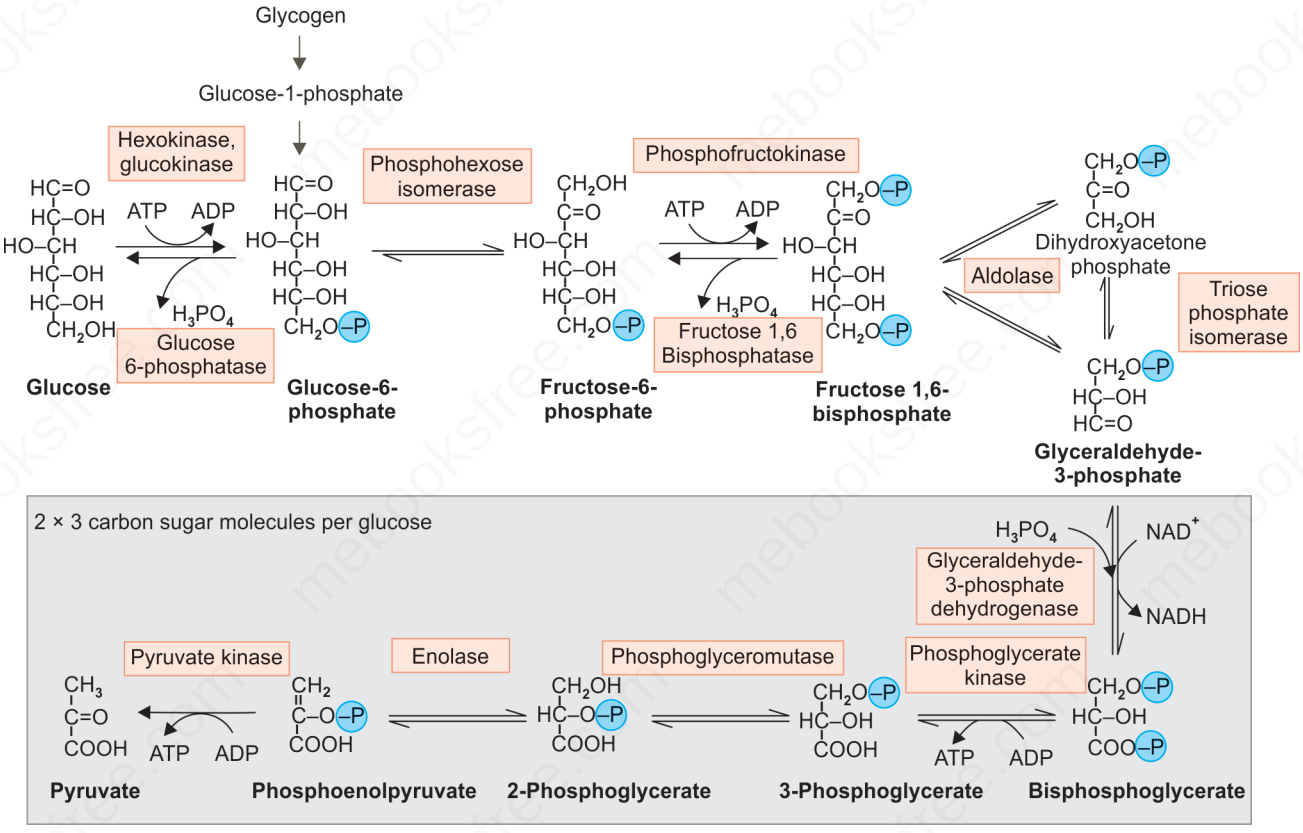


Fig. 5.1: Glycolysis

Biochemical Significances of Glycolysis

- Principal route for Carbohydrate metabolism
- Pathway taking place in all the cells of the body
- Only pathway which can operate aerobically and anaerobically
- The ability to operate glycolysis in the absence of oxygen is important in Skeletal muscle
- Skeletal muscles can survive anoxic episodes because of anaerobic glycolysis
- Defect in muscle Phosphofructokinase manifest as fatigue because of its significance in muscle
- Heart muscle has relatively low glycolytic activity, hence poor survival under conditions of ischemia
- Mature Erythrocyte which lack mitochondria are completely reliant on Glucose as their metabolic fuel[Q AIIMS May 2015]
- Defect in Glycolytic enzyme like Pyruvate Kinase manifest as hemolytic anemia, because of its significance in mature erythrocytes.

Overview of Glycolysis

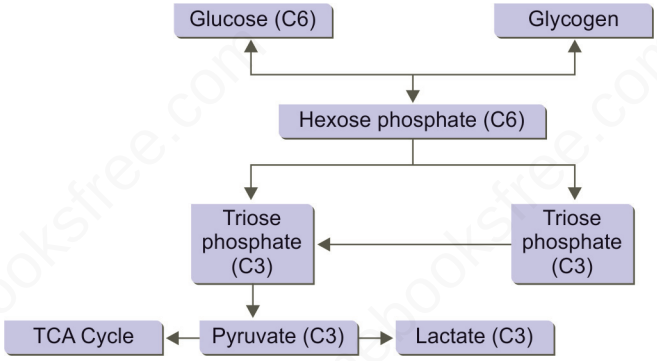


Fig. 5.2: Overview of gycolysis

Steps of Glycolysis

Site–Cytoplasm

Step 1-Hexokinase/Glucokinase

Hexokinase

- Transfer Phosphate group from ATP to Glucose
- Has high affinity for glucose (Or Lower Km)
- Mg^{2+} is the cofactor
- Irreversible step
- ATP is utilized.

Glucokinase

- Present in Liver and Pancreatic β cells
- Has low affinity for Glucose. (High Km)
- Hence acts only when blood glucose is very high (> 100 mg/dl)
- Induced by Insulin following a meal[Q]
- Play an important role in regulation of blood glucose.[Q]
- In the liver, function of Glucokinase is to remove glucose from portal vein following a meal
- In the beta cells of Pancreas, function of Glucokinase is to release Insulin.

Fates of Glucose 6 Phosphate

- HMP Shunt Pathway
- Gluconeogenesis
- ***Glycogenesis (Major fate in well fed state)*** [(AIIMS May 93)]
- Glycogenolysis.

Step II

- Isomerization of Glucose 6 Phosphate to Fructose 6 Phosphate by Phosphohexose Isomerase
- Involves Aldose–Ketose Isomerism

Step III

- Fructose 6 Phosphate is phosphorylated to Fructose 1, 6 Bisphosphate by Phosphofructokinase I (PFK-I)
- Second irreversible step
- Major regulatory step of Glycolysis[Q]
- Committed[Q] step of Glycolysis because once Fructose 1, 6 Bisphosphate is formed it should undergo glycolysis
- Otherwise called bottle neck of Glycolysis
- 1 ATP is utilized.

Step IV

- Fructose 1, 6 Bisphosphate split to 2,3 Carbon compounds Glyceraldehyde 3 Phosphate and Dihydroxyacetone phosphate
- By the Enzyme Aldolase
- Aldolase is a Lyase[Q].

Step V

- Dihydroxyacetone Phosphate isomerized to Glyceraldehyde 3 Phosphate by Triose Phosphate Isomerase.

Step VI

- Glyceradehyde 3 Phosphate is oxidized to a high energy compound, 1,3 bisphosphoglycerate
- By the enzyme Glyceraldehyde 3 Phosphate Dehydrogenase
- NADH is generated
- An inorganic Phosphate is added[NBE patternQ2013]
- NADH generated in this step enter into mitochondria by Malate –Aspartate Shuttle or Glycerophosphate shuttle under aerobic conditions
- But in anaerobic conditions NADH is utilized by Lactate Dehydrogenase, NAD + is regenerated.

Step VII
- 1,3 Bisphosphoglycerate to 3 Phosphoglycerate
- By the enzyme 1,3 Bisphosphoglycerate Kinase
- Only Kinase in Glycolysis which is reversible
- ATP is formed
- An example of Substrate level Phosphorylation[Q].

Step VIII
- 3 Phosphoglycerate to 2 Phosphoglycerate
- By Phosphoglycerate Mutase.

Step IX
- 2 Phosphoglycerate to Phosphoenol Pyruvate
- This step involves dehydration
- By the enzyme Enolase
- Enolase is dependent on Mn^{2+} and Mg^{2+}
- Fluoride inhibit Enolase.

Step X
- Phosphoenol Pyruvate to Pyruvate[Q]
- By Pyruvate Kinase
- Second substrate level Phosphorylation
- ATP is generated
- Irreversible step.

ANAEROBIC GLYCOLYSIS

- Pyruvate to Lactate by the enzyme Lactate Dehydrogenase
- NADH is utilized in this step
- NAD+ is regenerated.

Remember
Tissues that derive much of their energy from Glycolysis and produce lactate or in other words, tissues that depend mainly on Glucose as metabolic fuel
- White fibers of Skeletal muscle
- Mature Erythrocytes
- Brain
- Gastrointestinal Tract
- Renal Medulla
- Skin
- Many Cancer cells.

Irreversible Steps of Glycolysis[Q]
- Hexokinase
- Phosphofructokinase
- Pyruvate Kinase.

Remember
All the Kinases are irreversible except 1,3 Bisphosphoglycerate Kinase which is reversible.

Substrate Level Phosphorylation[Q]
- Phospho Glycerate Kinase [1,3 Bisphosphoglycerate to 3 Phosphoglycerate]
- Pyruvate Kinase [Phosphoenol Pyruvate to Pyruvate].

NB: Learn the enzyme and the reaction. Question can be asked in either ways.

Inhibitors of Glycolysis
- Iodoacetate inhibit Glyceraldehyde 3 Phosphate Dehydrogenase
- Fluoride inhibit Enolase
  - Application: For Estimation of Blood Glucose: Sodium Fluoride: Potassium Oxalate Mixture is used
- Arsenite is toxic to 1,3 Bisphosphoglycerate Kinase step.

CLINICAL CORRELATION–GLYCOLYSIS

- Inherited Aldolase A and pyruvate Kinase deficiency in erythrocyte leads to Hemolytic anemia
- Muscle Phosphofructokinase deficiency leads to Exercise Intolerance.

Energetics of Aerobic Glycolysis[Q]

Enzyme	Reducing equivalents/ ATP from the step	ATP per molecule of Glucose
Glyceraldehyde 3 Phosphate Dehydrogenase	NADH = 2.5 ATPs	2 NADH = 5ATPs
1, 3 Bisphospho-Glycerate Kinase	1 ATP by substrate level Phosphorylation	2 ATPs
Pyruvate Kinase	1 ATP by Substrate level Phosphorylation	2 ATPs
The number of ATPs generated		9 ATPs
Consumption of ATPs in the Hexokinase and Phosphofructokinase		-2ATP
No of ATPs from Aerobic Glycolysis		9–2 = 7 ATPs

Energetics of Anaerobic Glycolysis

Enzyme	Reducing equivalents/ ATP from the step	ATP per molecule of Glucose
1,3 Bisphosphoglycerate Kinase	1 ATP by Substrate level Phosphorylation	2 ATPs
Pyruvate Kinase	1 ATP by Substrate level Phosphorylation	2 ATPs
The number of ATPs generated		4ATPs
Consumption of ATPs in the HexoKinase and Phosphofructokinase		-2ATP
No of ATPs from Anaerobic Glycolysis		4–2 = 2 ATPs

Energy yield from 1 mol of Glucose under aerobic condition

Source	No of ATPs generated
From Aerobic Glycolysis	7 ATPs
From Pyruvate Dehydrogenase (as 2 Pyruvates from I mol of Glucose)	2 NADH = 5ATPs
From TCA Cycle (As 2 Acetyl CoA from I mol of Glucose)	2 × 10 = 20 ATPs
Net ATPs from 1 mol of Glucose under aerobic condition	7 + 5 + 20 = 32 ATPs
Net ATPs from 1 mol of Glucose under anaerobic condition	4 – 2 = 2 ATPs

Remember
- The number of ATPs produced from 1 NADH if Malate shuttle is used for transport of NADH into mitochondria is 2.5 ATPs.
- The number of ATPs produced from 1 NADH if Glycerophosphate shuttle is used for transport of NADH into mitochondria is only 1.5 ATPs.
- If Muscle Glycogen is used for anaerobic glycolysis, then 3 ATPs are produced instead of 2 ATPs because, as there is no Glucose 6 Phosphatase in muscle Glucose 6 Phosphate directly enter into Glycolysis. Hence 1 ATP for Hexokinase step is not needed. So out of 4 ATPs produced only 1 is utilized, resulting in 3 ATPs from 1 mol of Glucose.

Key Concept of regulation of all metabolic pathways

Hormonal regulation
- **Insulin** generally favor all pathways which decrease blood glucose level by **dephosphorylating** the regulatory enzymes of these pathways
- In other words enzymes active under the influence of insulin is active in the dephosphorylated state
- **Glucagon** generally favor all pathways which increase blood glucose level by **phosphorylating** the regulatory enzymes of these pathways
- In other words enzymes active under the influence of glucagon is active in the phosphorylated state.

Allosteric Regulation
- Substrate favor forward reaction
- Product inhibits forward reaction.

REGULATION OF GLYCOLYSIS

Glycolysis is regulated at three physiologically irreversible steps

1. Hexokinase/Glucokinase
2. Phosphofructokinase-I (Occupies a key position in the regulation of Glycolysis)
3. Pyruvate Kinase.

Hormonal Regulation

Insulin favor glycolysis
- By dephosphorylating key enzymes of Glycolysis
- By Inducing Glucokinase
- Glucokinase play a key role in regulating blood glucose level after a meal.

Glucagon inhibit glycolysis
- Increasing cAMP dependent Protein Kinase A
- By Phosphorylating key Enzymes of Glycolysis.

Allosteric Regulation[Q] DNB 2000

Enzyme	Allosteric activator	Allosteric inhibitor
Hexokinase		Glucose-6-phosphate
PFK-1	5' AMP Fructose-6- Phosphate, Fructose 2,6 Bisphosphate.	ATP[Q] Citrate[Q] Low pH
Pyruvate Kinase		ATP

Remember
- PFK-1 plays a key role in regulation of Glycolysis
- Glucokinase play a key role in regulating blood glucose level following a meal.

RAPAPORT-LEUBERING CYCLE (2,3 BPG SHUNT)

- **Site:** Mature erythrocytes
- The reaction catalyzed by phosphoglycerate kinase may be bypassed
- 1,3-bisphosphoglycerate is converted to 2,3-bisphosphoglycerate by bisphosphoglycerate 2,3- bisphosphoglycerate
- 2,3 Bisphosphoglycerate is hydrolyzed to 3-phosphoglycerate and Pi by 2,3-bisphosphoglycerate phosphatase mutase
- No ATP is generated by this step
- 2 ATPs at Pyruvate Kinase step is generated but that is used for Hexokinase and Phosphofructokinase
- So no net yield of ATPs 2,3 BPG shunt pathway
- Serve to provide 2,3-bisphosphoglycerate[Q]
- 2,3 BPG shifts the oxygen Dissociation curve to right.

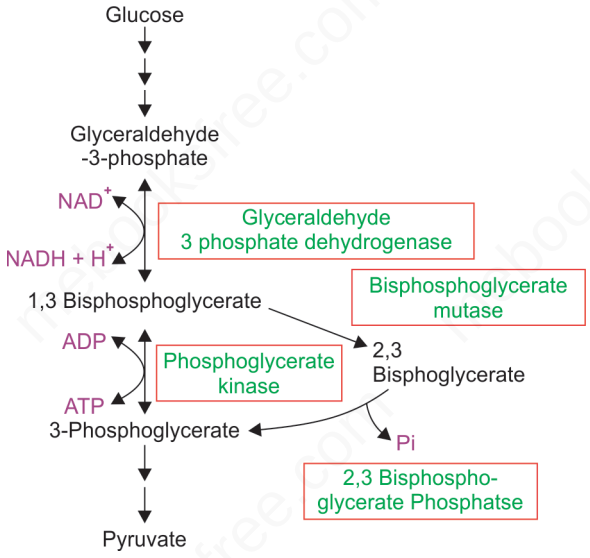


Fig. 5.3: Rapaport-Leubering cycle

Points to Ponder

Net no of ATPs produced from 1 mol of Glucose by

- Anaerobic Glycolysis-2 ATPs
- Aerobic Glycolysis-7 ATPs
- Aerobic oxidation-32 ATPs
- Rapaport-Leubering Cycle-0

Cancer Cells and Metabolic Reprogramming

A comparison of Warburg effect and recent data about altered metabolism in cancer cells Warburg effect

In 1924, Otto Warburg and his colleagues observed that cancer cells take up large amount of glucose and metabolize it to lactic acid even in presence of oxygen. This observation is called Warburg effect.

The hypothesis made by him based on this data were:

- Increased ratio of glycolysis when compared to aerobic respiration was likely due to defect in mitochondrial respiratory chain
- Enhanced glycolysis help cancer cells to preferentially proliferate in reduced oxygen tension
- He also argued that switch from aerobic to anaerobic glucose metabolism was the driver of tumorigenesis.

Recent data-Metabolic reprogramming of cancer cells

Rather than overt defect in mitochondrial respiratory chain, a metabolic reprogramming is typically observed in tumor cells.

Metabolic Enzyme reprogramming

Genetic changes in specific metabolic enzyme encoding gene, preferentially express mRNA splice variants. The mutated enzymes produce oncometabolites, that regulate gene expression by epigenetic mechanism.

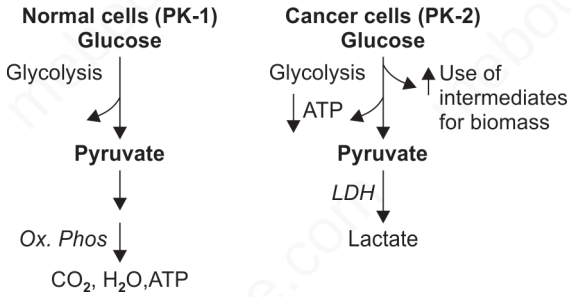
Metabolic reprogramming of Glycolysis result in

- Less shuttling of glucose –derived chemical energy by Glycolysis into production of ATP
- Shunting of glucose derived chemical energy for building up of cellular biomass of proteins, lipids, etc.

These explain the increased rate of glycolysis in tumor cells.

Increased anaerobic Glycolysis in tumor cells despite angiogenesis can be explained by:

- Despite angiogenesis there is local areas of poor blood supply
- This results in low oxygen tension
- Induction of Hypoxia-inducible factor-1 (HIF-1)
- Upregulate the genes controlling glycolysis.



Clinical application of metabolic reprogramming

- *Early detection of cancer:* Blood and urine mass spectrometry to look for altered metabolic profile helps in the early detection of cancer
- *Development of anticancer drugs:* Chemicals that inhibit glycolysis will selectively kill cancer cells.

Compound	Enzyme inhibited
3 Bromo-pyruvate	Hexokinase II
2 Deoxy d glucose	Hexokinase I
Dichloroaceate	Pyruvate Dehydrogenase Kinase
Iodoacetate	Glyceraldehyde 3 Phosphate dehydrogenase

FATES OF PYRUVATE

- To Glucose (Gluconeogenesis)
- To Lactate (Lactate Dehydrogenase)
- To Oxaloacetate (Pyruvate Carboxylase)
- To Acetyl CoA (Pyruvate Dehydrogenase)
- To Alanine (Alanine Amino Transferase).

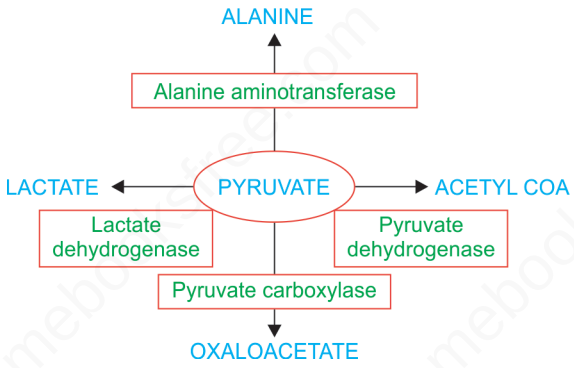


Fig. 5.4: Fates of pyruvate

PYRUVATE DEHYDROGENASE (PDH) COMPLEX

- Pyruvate formed in the cytosol enter the mitochondria by a symporter

- Pyruvate is oxidatively decarboxylated to Acetyl CoA
- The oxidation of Pyruvate to Acetyl-CoA is the irreversible route from glycolysis to the citric Acid Cycle
- No alternate pathway to circumvent this step
- Pyruvate dehydrogenase complex is analogous to the α-ketoglutarate dehydrogenase
- Multienzyme complex associated with the inner mitochondrial membrane.

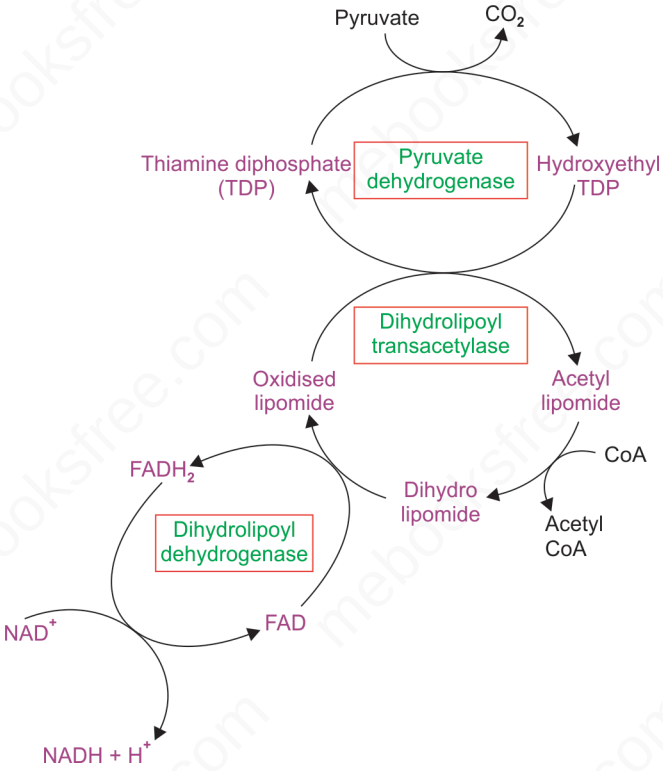


Fig. 5.5: Pyruvate dehydrogenase complex

PDH complex consist of 3 Enzymes and 5 Coenzymes
Three enzymes are

- Pyruvate Dehydrogenase bound to Thiamin Diphosphate
- Dihydrolipoyl Transacetylase, the prosthetic group is oxidised Lipomide
- Dihydrolipoyl Dehydrogenase, contains FAD.

Coenzymes[Q] (Very important)

- Thiamine Diphosphate (TDP)
- Lipomide
- Coenzyme A
- FAD
- NAD^+

Regulation of Pyruvate Dehydrogenase

By end product inhibition and covalent modification

End products that inhibit PDH Complex are

- Acetyl CoA
- NADH

By Covalent modification

PDH is active in dephosphorylated state and inactive in phosphorylated state

PDH is phosphorylated by a PDH Kinase

PDH Kinase is activated by increase in:

- ATP/ADP
- Acetyl CoA/CoA
- $NADH/NAD^+$

PDH is dephosphorylated by PDH Phosphatase

- ***Insulin favor PDH Phosphatase, hence dephosphorylate PDH Complex and PDH is active.***

Significance of PDH Complex

- Thiamine deficiency affect PDH. Hence complete oxidation of Glucose
- PDH defect can lead to Lactic Acidosis[Q]
- Fat cannot be converted to Glucose because of the irreversible nature of PDH
- **Acetyl CoA[Q] cannot be converted to glucose.**

Exception to fat cannot be converted to Glucose

- Glycerol part of Triacyl Glycerol
- Odd Chain Fatty Acid oxidation which forms Propionyl CoA.

Fate of Acetyl CoA (AI 09, DNB 08, 09, AIIMS May 03)

- Fatty Acid Synthesis
- Ketone Body Synthesis
- Cholesterol Synthesis
- TCA Cycle

Acetyl CoA cannot be converted to glucose.[Q]

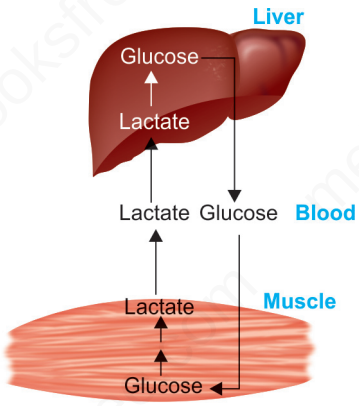


Fig. 5.6: Cori's cycle

Cori's Cycle (Glucose-Lactate Cycle) (Lactic Acid Cycle)

Lactate, formed by glycolysis in skeletal muscle and erythrocytes, is transported to the liver and kidney where it reforms glucose, which again becomes available via the circulation for oxidation in the tissues. This process is known as the Cori cycle, or the lactic acid cycle.

Uses

- Prevents Lactate accumulation in the muscle
- Reutilize lactate from muscle and erythrocyte for Gluconeogenesis.

Cori's cycle involves

- Liver and Kidney
- Muscle
- RBC.

Glucose Alanine Cycle (Cahill Cycle)

In the fasting state, there is a considerable output of alanine from skeletal muscle formed by transamination of pyruvate produced by glycolysis of muscle glycogen, and is exported to the liver, where, after transamination back to pyruvate, it is a substrate for gluconeogenesis. This is **glucose-alanine cycle.** It provides an indirect way of utilizing muscle glycogen to maintain blood glucose in the fasting state.

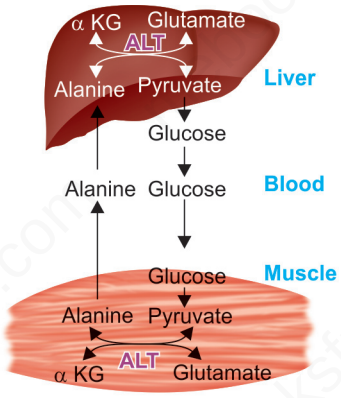


Fig. 5.7: Glucose alanine cycle

Uses of glucose alanine cycle

- Carries amino group to the Liver
- Alanine as a substrate for Gluconeogenesis during starvation
- Amino acid increased in blood during starvation is Alanine.[AIIMS Nov 2011]

GLUCONEOGENESIS

Definition: The process of formation of ***glucose or Glycogen*** from noncarbohydrate precursors.

Substrates for Gluconeogenesis[Q] (very important)

- Glucogenic Amino Acid [Alanine[Q] is the major contributor]
- Lactate
- Glycerol
- Propionyl CoA.

Sites of Gluconeogenesis[Q]

Liver[Q] (60–90%) Kidney[Q] (10–40%)

Organelle[Q]-Cytoplasm and Mitochondria

Remember

Key Gluconeogenic enzymes are expressed in the small intestine but it is unclear that significant Gluconeogenesis in intestine during starvation.

Biomedical Significance of Gluconeogenesis

- Provides a major contribution to blood glucose after overnight fast, once Glycogen stores are depleted
- A supply of glucose is essential for erythrocytes and brain
- Glucose is important for maintaining the intermediates of Citric Acid Cycle
- Gluconeogenesis clears the lactate produced in the erythrocytes and skeletal muscle and glycerol produced in the adipose tissue
- Excessive gluconeogenesis occur in critically ill patients in response to injury and infection
- Excessive gluconeogenesis is contributory factor to hyperglycemia in type II Diabetes mellitus
- Involves Glycolysis, the Citric Acid Cycle, plus some special reactions.

GLUCONEOGENESIS–STEPS

1. Pyruvate and Phosphoenolpyruvate

Reversal of the reaction catalyzed by pyruvate kinase in glycolysis involves two endothermic reactions.

- Pyruvate Carboxylase
- Phosphoenolpyruvate Carboxykinase (PEPCK).

Pyruvate Carboxylase

- Mitochondrial pyruvate carboxylase catalyzes the carboxylation of Pyruvate to Oxaloacetate
- It is an ATP-requiring reaction
- Biotin is the coenzyme
- The resultant oxaloacetate is reduced to malate, exported from the mitochondrion into the cytosol and there oxidized back to oxaloacetate.

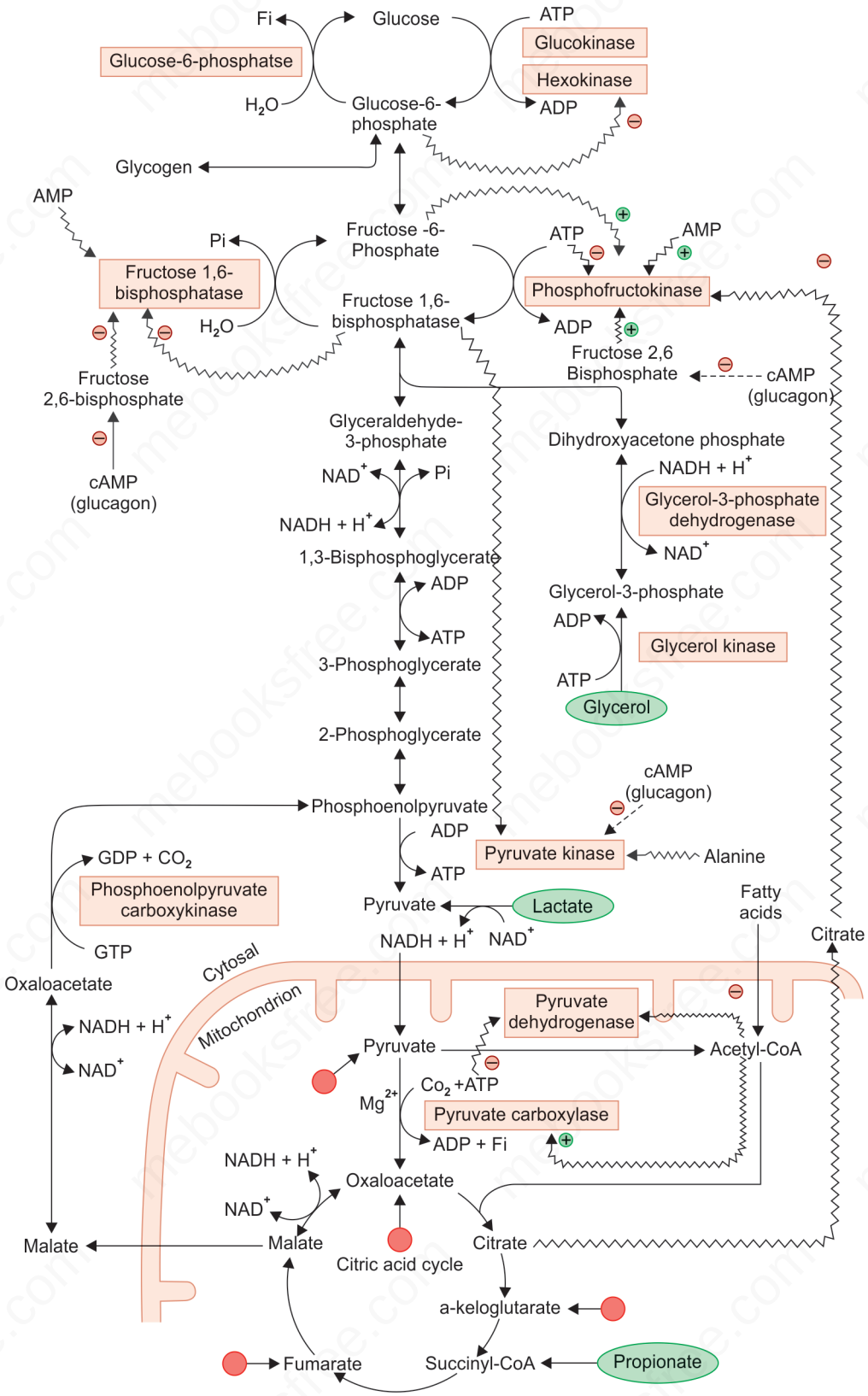


Fig. 5.8: Gluconeogenesis

Phosphoenolpyruvate Carboxykinase

- Catalyzes the decarboxylation and phosphorylation of oxaloacetate to phosphoenolpyruvate using GTP as the phosphate donor.

2. Fructose 1,6-Bisphosphate and Fructose 6-Phosphate

Fructose 1,6 Bisphosphatase

- The conversion of fructose 1,6-bisphosphate to fructose 6-phosphate, for the reversal of glycolysis, is catalyzed by fructose 1,6-bisphosphatase.

3. Glucose 6-Phosphate and Glucose

Glucose 6 Phosphatase

The conversion of glucose 6-phosphate to glucose is catalyzed by glucose 6-phosphatase.

It is present in liver and kidney, but **absent from muscle and adipose tissue**, which, therefore, cannot export glucose into the bloodstream.

Glucose 1-Phosphate and Glycogen

Glycogen synthesis involves a different pathway via uridine diphosphate glucose and glycogen synthase.

Summary of Key Enzymes of Gluconeogenesis

Irreversible steps of glycolysis	Key enzymes to bypass the irreversible steps in gluconeogenesis
1. Pyruvate Kinase Convert Phosphoenol Pyruvate to Pyruvate	Pyruvate Carboxylase (Mitochondria) Convert Pyruvate to Oxaloacetate Oxaloacetate transported to the Cytosol by Malate Shuttle[Q] Phosphoenol Pyruvate Carboxy Kinase (PEPCK) (Cytosol) Convert Oxaloacetate to Phosphoenol Pyruvate
2. Phosphofructokinase	Fructose 1,6 Bisphosphatase [Cytosol]
3. Hexokinase/Glucokinase	Glucose 6 Phosphatase [Cytosol]

Entry of Propionyl CoA to Gluconeogenesis

By three enzymes

1. Propionyl CoA Carboxylase
2. Methyl Malonyl CoA Racemase
3. Methyl Malonyl CoA Mutase

- Propionyl-CoA is carboxylated to D-methyl-malonyl-CoA, catalyzed by **propionyl-CoA carboxylase,** a biotin-dependent enzyme
- **Methylmalonyl-CoA racemase** catalyzes the conversion of D-methylmalonyl-CoA to L-methylmalonyl-CoA
- Then undergoes isomerization to succinyl-CoA catalyzed by **methylmalonyl-CoA mutase, vitamin B_{12} – dependent enzyme.**

Sources of Propionyl CoA in Humans

- β oxidation of Fatty acid
- Oxidation of Isoleucine
- Side chain of Cholesterol.

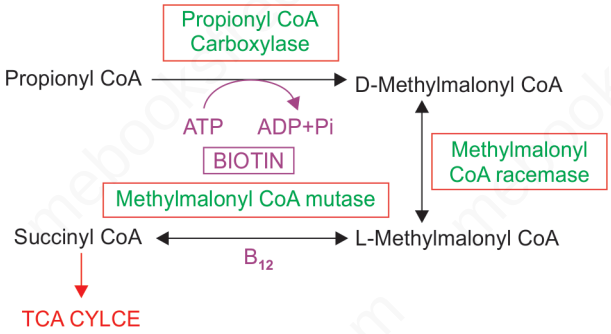


Fig. 5.9: Entry of propionyl CoA to gluconeogenesis

Clinical Correlation

Methylmalonic aciduria

Methylmalonyl-CoA mutase is a vitamin B_{12}-dependent enzyme, and in deficiency methylmalonic acid is excreted in the urine (methylmalonic aciduria)

- **Enzymes Common to Glycolysis and Gluconeogenesis**[(AI 97, Kerala 2007)]
- All the enzymes other than the irreversible enzymes in the glycolysis
- One mol of lactate converted to Glucose-6 ATPs are utilized.

RECIPROCAL REGULATION OF GLUCONEOGENESIS AND GLYCOLYSIS

Since Glycolysis and Gluconeogenesis share the same pathway but in opposite directions, they must be regulated reciprocally.

Three mechanisms are responsible for regulating the activity of enzymes concerned in carbohydrate metabolism:

1. Changes in the Rate of Enzyme Synthesis—By Induction and Repression

- Insulin, secreted in response to increased blood glucose, enhances the synthesis of the key enzymes in glycolysis

- Pyruvate Carboxylase is repressed by Insulin (DNB 09, AIIMS May 2013
- Insulin also antagonizes the effect of the glucocorticoids and glucagon-stimulated cAMP, which induce synthesis of the key enzymes of gluconeogenesis.

2. Covalent Modification by Reversible Phosphorylation–By means of Hormones

Epinephrine and Glucagon

- Increasing the concentration of cAMP
- This in turn activates cAMP-dependent protein kinase
- Leading to the phosphorylation and inactivation of pyruvate kinase.

Insulin

- Decrease the concentration of cAMP
- Dephosphorylate the key enzymes of Gluconeogenesis and become inactive.

3. Allosteric Modification

By Acetyl CoA and Fructose 2,6 Bisphosphate

Acetyl CoA

- Acetyl-CoA as an allosteric activator[QQQ] of Pyruvate Carboxylase.
- This ensures provision of Oxaloacetate, so that Acetyl CoA can be oxidised by Citric acid cycle.

Fructose 2,6 Bisphosphate

First we learn about the tandem enzyme that synthesize Fructose 2,6 Bisphosphate.

Tandem Enzyme (Bifunctional Enzyme)

- Single polypeptide with two enzyme activity
- Two enzyme activities are Phosphofructokinase–II (PFK-II) and Fructose 2,6 Bisphosphatase (F2,6 BPase).

Action of the tandem enzyme

- PFK-II Convert Fructose 6 Phosphate to Fructose 2,6 Bisphosphate
- F2, 6 BPase Convert Fructose 2,6 Bisphosphate to Fructose 6 Phosphate
- ***Fructose 2,6 Bisphosphate, the product of PFK-II, is an allosteric activator of PFK-I***

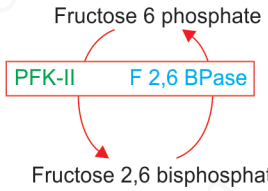


Fig. 5.10: Action of bifunctional enzyme that regulate glycolysis and gluconeogenesis

In Well Fed State

- Insulin dephosphorylate the tandem enzyme
- PFK-II part is active and F2, 6 BPase is inactive
- Level of Fructose 2,6 Bisphosphate rises
- This favors Glycolysis
- But Gluconeogenesis is inactive
- Decreases the blood Glucose.

In the Fasting State

- Glucagon phosphorylate the tandem enzyme by cAMP dependent Protein Kinase
- PFK-II is inactive and F2,6 BPase is active
- Level of Fructose 2,6 Bisphosphate falls
- This favors Gluconeogenesis
- But Glycolysis is inactive
- Increases the blood Glucose.

Fructose 2, 6 Bisphosphate reciprocally regulate Glycolysis and Gluconeogenesis

Characteristics	Phosphofructo-kinase-II	Fructose 2,6 Bisphosphatase
Reaction	Fructose 6 Phosphate to Fructose 2,6 Bisphosphate	Fructose 2,6 Bisphosphate to Fructose 6 Phosphate Remember the reverse reaction of PFK-II
Hormonal regulation	Favored by Insulin	Favored by Glucagon
Covalent Modification	Active in dephosphorylated state	Active in Phosphorylated state.
Dietary regulation	Active in well fed state	Active in fasting state.
Reciprocal regulation of Glycolysis & Gluconeogenesis	Fructose 2,6 Bisphosphate, the product of PFK-II favor Glycolysis. inhibit Gluconeogenesis	Decreases the level of Fructose 2,6 Bisphosphate, there by favor Gluconeogenesis inhibit Glycolysis.

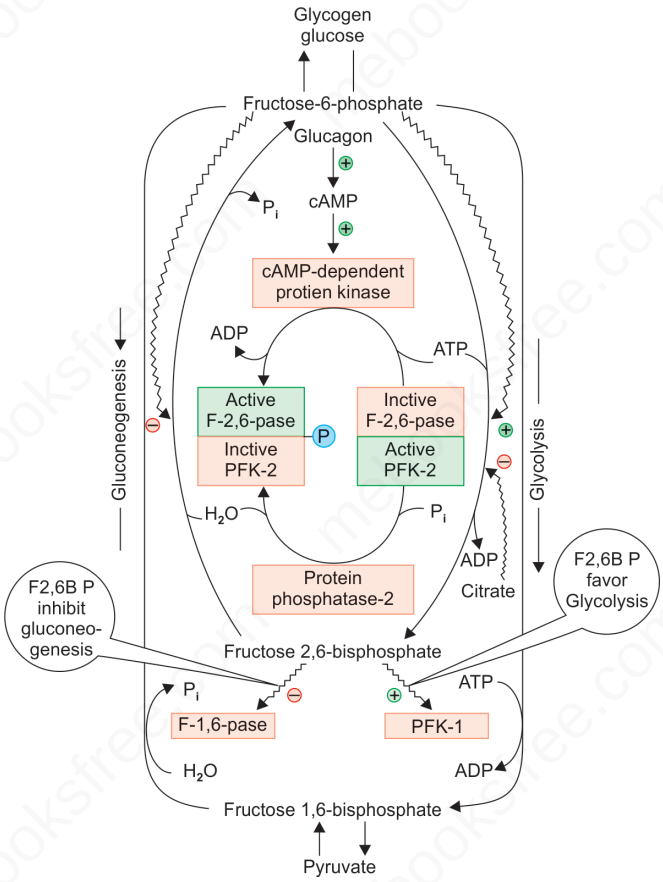


Fig. 5.11: Reciprocal regulation of glycolysis and gluconeogenesis

GLYCOGEN METABOLISM

- **Glycogen Synthesis (Glycogenesis)**
- **Glycogen Degradation (Glycogenolysis)**
  - Glycogen is the major storage carbohydrate in animals
  - Glycogen is present mainly in liver and muscle, with modest amount in brain.

Structure of Glycogen

- Branched homopolysaccharide made up of α D Glucose.

Linkage

- α 1,4 linkage at unbranched points
- α 1,6 linkage at branching points.

Differences between liver glycogen and muscle glycogen

Features	Liver	Muscle
Total Glycogen content	Less (1.8 Kg)	Highest (35 Kg)
Percentage by tissue weight	Highest (5.0)	Less (0.7)

Contd...

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Features	Liver	Muscle
Regulation of blood glucose	Contributes to blood glucose	Does not directly contribute to blood glucose. But serves as a source of energy to muscle itself
Glucose 6 Phosphatase	Present	Absent [Q]

Remember
After 12–18 hour of fasting, liver glycogen is almost totally depleted.[Q]

Muscle Glycogen and Gluconeogenesis
Muscle does not contribute directly to blood Glucose but Pyruvate formed by Glycolysis, is transaminated to Alanine. This is transported to Liver, which is used for Gluconeogenesis. This is *Glucose Alanine Cycle*.

GLYCOGENESIS

- Occurs mainly in Muscle and Liver
- Organelle-Cytosol
- Rate Limiting Enzyme: Glycogen Synthase.

Glycogenesis–Steps

Synthesis of UDP glucose

- Glucose converted to Glucose 6 Phosphate by Hexokinase in muscle/Glucokinase in liver
- Glucose 6 Phosphate isomerized to Glucose 1 Phosphate Phosphoglucomutase
- Glucose 1 Phosphate react with UTP to form UDP Glucose and Pyrophosphate catalyzed by UDP Glucose PyroPhosphorylase
- UDP Glucose is the Glucose donor for Glycogen Synthesis.

Initiation of glycogen synthesis

- Glycogen Synthase is the enzyme that joins Glucose residues by α 1,4 Linkage (C1 of UDP Glucose and C- 4 of the terminal glucose residue in the Glycogen, liberating UDP)
- But Glycogen Synthase can do this only on a pre-existing Glycogen molecule or a primer called Glycogenin
- Glycogen Synthase adds Glucose residue on this Glycogen Primer.

Glycogenin

Glycogenin is a 37 kDa protein[QNBE pattern] that is glucosylated on a specific tyrosine residue by UDPGlc.

Formation of branch points

- When the chain is at least 11 glucose residues long, branching enzyme acts
- Branching enzyme transfers at least six glucose residues to a neighboring chain to form a α 1,6 linkage, establishing a branch point
- Branches grow by further addition of α 1 → 4 glucosyl units.

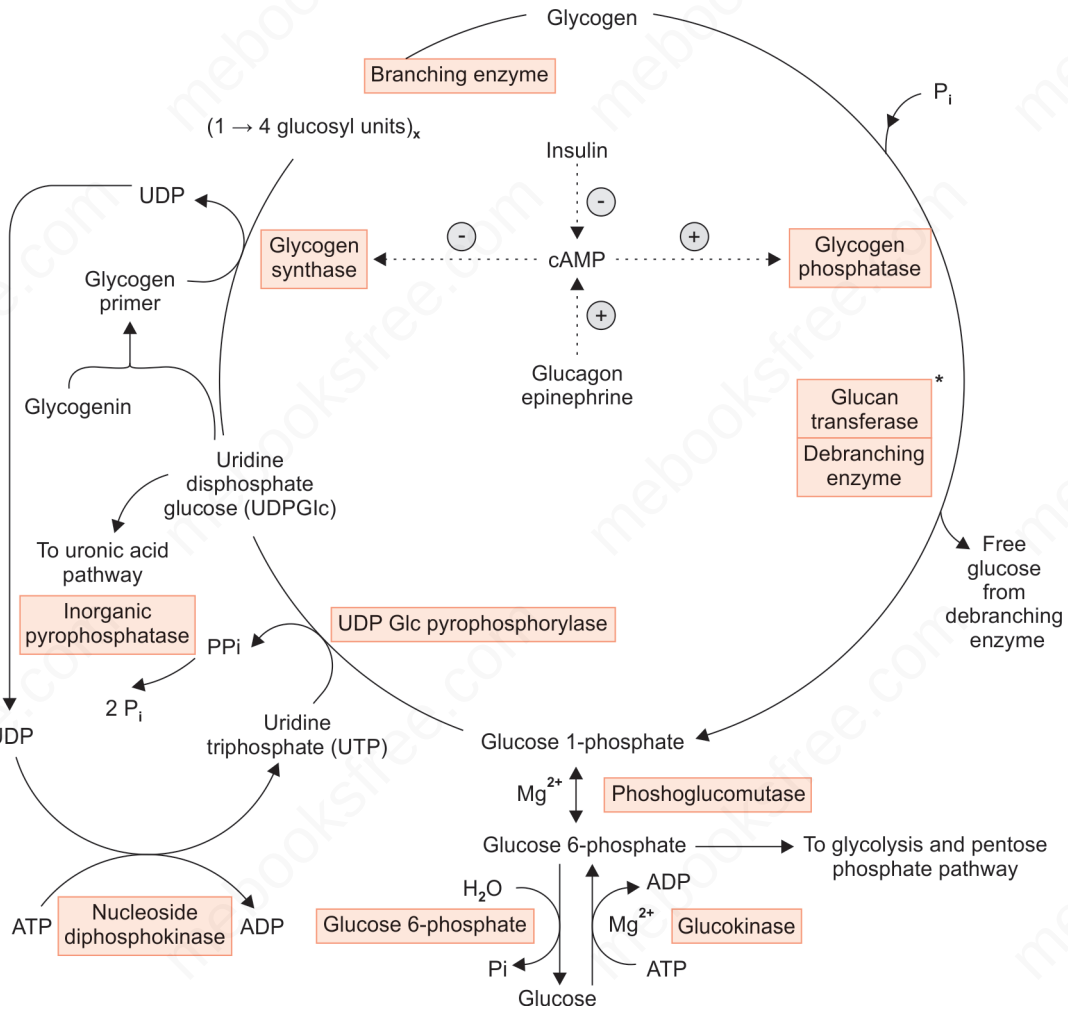


Fig. 5.12: Pathways of glycogenesis and glycogenolysis

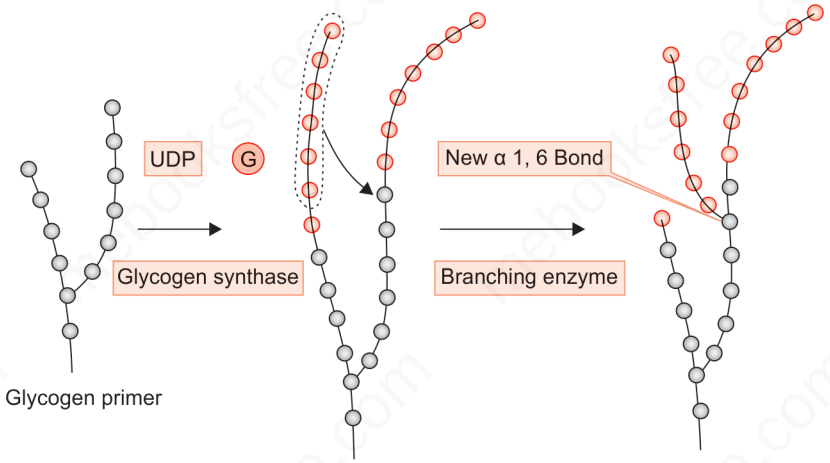


Fig. 5.13: Synthesis of glycogen

GLYCOGENOLYSIS

- Occurs in the Muscle and Liver
- Organelle Mainly Cytoplasm [Small proportion in the Lysosomes]
- Rate Limiting Enzyme-Glycogen Phosphorylase
- PLP is a Coenzyme[Q] of Glycogen Phosphorylase.

Isoenzymes of Glycogen Phosphorylase
- Present in Muscle, Liver and Brain.
- Glycogen Phosphorylase BB is a Cardiac Biomarker.

Steps of Glycogenolysis

Breaking of α 1, 4 linkage

- Glycogen Phosphorylase cleave the **α 1,4 linkage**
- Releases **Glucose 1 Phosphate NOT free Glucose**
- Glycogen Phosphorylase stops its action when it is atleast 4 glucose residues from a branch point.

Removal of Branches

By a bifunctional enzyme:

- **First part is a α-1, 4 α 1,4 Glucan transferase**
  - Transfer trisaccharide residue to another forming a new α 1, 4 linkage.
- Second part is a α1,6 **Glucosidase (Amylo 1,6 Glucosidase)**
  - Hydrolyse the branching point
  - Releases free Glucose NOT Glucose 1 Phosphate.

Conversion of Glucose 1 Phosphate to free Glucose

- Glucose 1 Phosphate to Glucose 6 Phosphate by Phosphoglucomutase
- Glucose 6 Phosphate to Glucose by Glucose 6 Phosphatase
- Glucose 6 Phosphatase is present in the smooth endoplasmic reticulum
- A transporter is required for the transport of Glucose 6 Phosphate from SER to cytoplasm
- Defect in the Glucose 6 Phosphate transporter lead to Type Ib Glycogen Storage disorder.

Minor Pathways of Glycogenolysis

- Taking place inside **lysosomes**
- By the enzyme **Acid maltase**
- Glycogen is hydrolyzed to Glucose
- This is important in glucose homeostasis in **neonates.**
- Genetic lack of Lysosomal acid Maltase lead to **Type II Glycogen Storage Disorder (Pompe's Disease or Type II GSD)**

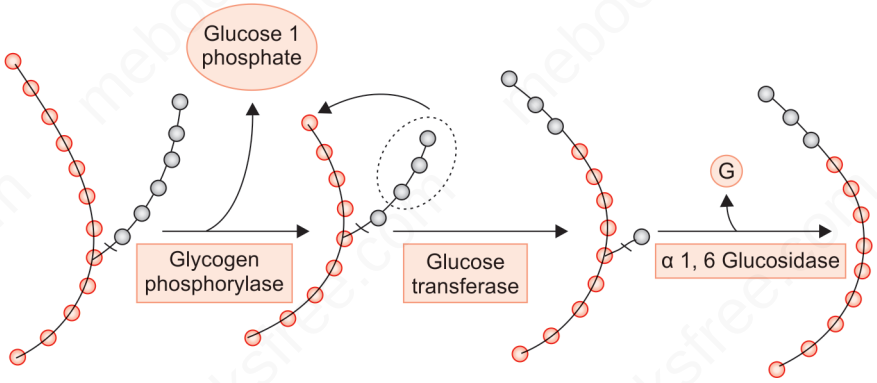


Fig. 5.14: Glycogenolysis

Remember
Enzyme common to Glycogenesis and Glycogenolysis is Phosphoglucomutase[Q] (AIIMS May 2014)

Regulation of Glycogen Metabolism

Basic Concepts

- Insulin favor Glycogenesis by dephosphorylating, Glycogen Synthase.
- Glycogen Synthase active in dephosphorylated state.
- Glucagon and Epinephrine favor Glycogenolysis by phosphorylating Glycogen Phosphorylase.
- Glycogen Phosphorylase active in the phosphorylated state.
- Phosphorylase a: Active state
- Phosphorylase b: Inactive state.

Rate Limiting Steps

- ***Glycogenesis:*** **Glycogen Synthase**
- ***Glycogenolysis:*** **Glycogen Phosphorylase.**

Hormonal Regulation

Insulin

- Insulin dephosphorylate Glycogen Synthase and Glycogen Phosphorylase
- Glycogen Synthase is active in the dephosphorylated state
- Glycogen Phosphorylase is inactive in the dephosphorylated state
- So Glycogen is synthesized.

Glucagon (In Liver) and Epinephrine (In liver and Muscle)

- Phosphosphorylate Glycogen Phosphorylase and Glycogen Synthase
- Glycogen Phosphorylase active in the phosphorylated state
- Glycogen Synthase inactive in the phosphorylated state
- So, Glycogen is degraded.

Remember

- Well fed state under the influence of Insulin store excess carbohydrate as Glycogen
- In fasting state under the influence of Glucagon, Glycogenolysis takes place in the liver to supply Glucose

Contd...

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- In an exercising muscle, epinephrine favor Glycogenolysis for supplying energy to the muscle
- Epinephrine has action only in the Muscle.

Differences between muscle and liver in the regulation of glycogen metabolism

- Epinephrine acts in Muscle and Liver where as Glucagon acts only in the Liver
- In the muscle there is **cAMP-independent activation of glycogenolysis**[Q]
  - By the stimulation of a Ca^{2+}/calmodulin-sensitive phosphorylase kinase
  - Phosphorylate Glycogen Phosphorylase in the muscle
  - Favor glycogenolysis.
- **Muscle phosphorylase can be activated without phosphorylation.**
  - Muscle Phosphorylase has a binding site for 5′AMP. 5′AMP is an allosteric activator without phosphorylation
  - Favor Glycogenolysis.

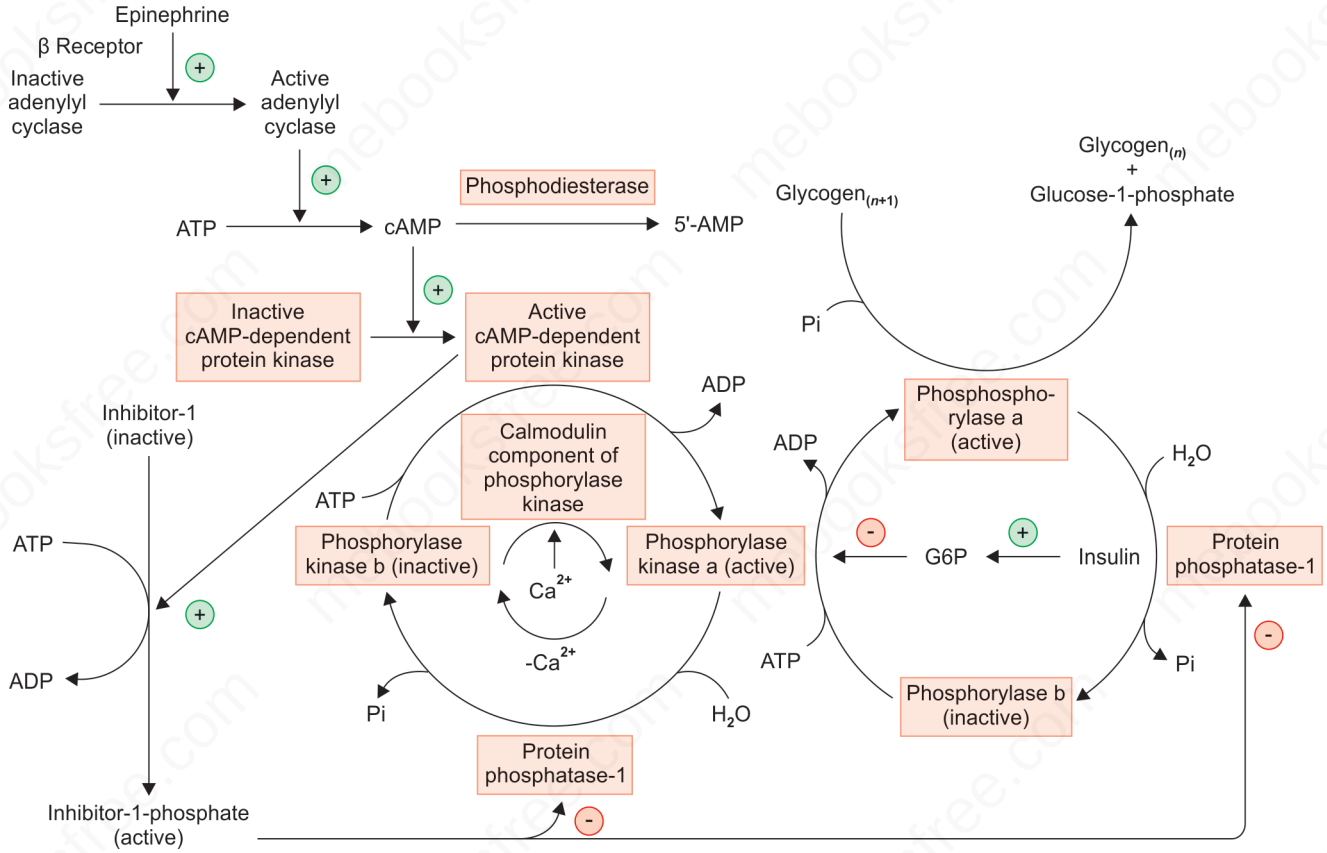


Fig. 5.15: cAMP dependent and cAMP independent mechanism regulation of glycogenphosphorylase

Mechanism of action of Glucagon and Epinephrine on Glycogen Metabolism

- Glucagon/Epinephrine bind to its receptor
- Inactive Adenylyl Cyclase is converted to Active Adenylyl Cyclase
- Adenylyl Cyclase convert ATP to cAMP
- cAMP activate inactive Protein Kinase A to active Protein Kinase A
- Phosphorylate Phosphorylase Kinase
- Phosphorylase Kinase b (Inactive) is now Phosphorylase Kinase a (Active)
- This Phosphorylate Glycogen Phosphorylase
- Glycogen Phosphorylase b (Inactive) is now Glycogen Phosphorylase a (Active)
- Glycogen is degraded (Ref. Figure 5.11).

Mechanism of Action of Insulin on Glycogen Metabolism

- Insulin increases the activity of Phosphodiesterase, which hydrolyses cAMP to 5′ AMP
- Thus insulin terminate the action of cAMP
- Increase the activity of Protein Phosphatase
- This dephosphorylate Glycogen Synthase and Phosphorylase Kinase
- So Glycogen Synthase is active
- Glycogen Phosphorylase is inactive
- Hence, glycogen Synthesis takes place.

ALLOSTERIC REGULATION OF GLYCOGEN SYNTHASE

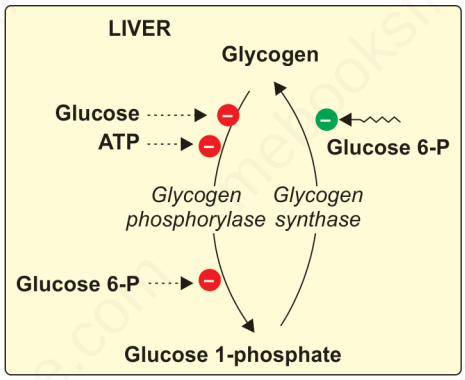


Fig. 5.16 *(Contd...)*

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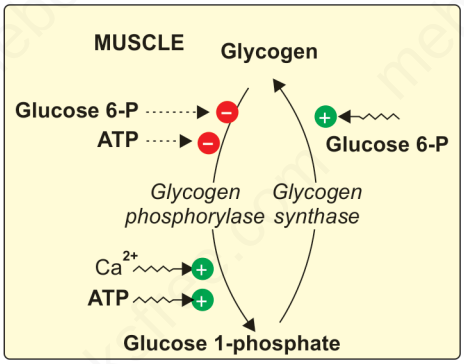


Fig. 5.16: Allosteric regulation of glycogen metabolism in muscle and liver

Allosteric regulators of Glycogen synthase and Glycogen phosphorylase

Organ	Enzyme	Allosteric Activator	Allosteric inhibitor
Liver	Glycogen Synthase	Glucose 6 Phosphate	----
	Glycogen Phosphorylase	-----	Glucose Glucose 6 Phosphate, ATP
Muscle	Glycogen Synthase	Glucose 6 Phosphate	-----
	Glycogen Phosphorylase	Ca++ AMP	Glucose 6 Phosphate, ATP

GLYCOGEN STORAGE DISORDERS (VERY IMPORTANT TOPIC)

Group of inherited disorders characterized by deposition of an abnormal type or quantity of glycogen in tissues, or failure to mobilize glycogen.

Common Glycogen Storage Disorders

Liver Glycogen Storage Disorder

Type	Name	Enzyme efficiency	Characteristics
0	——	Glycogen synthase	Early morning drowsiness and fatigue, fasting hypoglycemia, and ketosis; early death [No Hepatomegaly]
Ia	Von Gierke's disease	Glucose 6-phosphatase[a]	Glycogen accumulation in liver and renal tubule cells (Kidney Enlarged) hypoglycemia; elevated blood lactate, cholesterol, triglyceride, and uric acid levels

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Type	Name	Enzyme efficiency	Characteristics
Ib	——	Endoplasmic reticulum glucose 6-phosphate transporter	Same as type Ia, with additional findings of neutropenia and impaired neutrophil function, Recurrent Bacterial Infection, Inflammatory Bowel Disease
III	Limit dextrinosis, Forbe's or Cori's disease	Liver and muscle debranching enzyme (Amylo 1,6 Glucosidase)	Fasting hypoglycemia hepatomegaly in infancy accumulation of characteristic branched polysaccharide (limit dextrin) muscle weakness, elevated transaminase levels; liver symptoms can progress to liver failure later in life
IV	Amylopectinosis, Andersen's disease	Branching enzyme	Hepatosplenomegaly Accumulation of polysaccharide with few branch points Failure to thrive, hypotonia, hepatomegaly, splenomegaly, progressive cirrhosis (death usually before 5th yr), elevated transaminase levels
VI	Hers' disease	Liver phosphorylase	Hepatomegaly
VIII		Liver phosphorylase kinase	
	Fanconi Bickel Syndrome QNBE pattern	Glucose transporter 2 (GLUT-2)	Failure to thrive, rickets, hepatorenomegaly, proximal renal tubular dysfunction, impaired glucose and galactose utilization

Type Ia GSD-Von Gierke's Disease

- Most common Glycogen Storage Disorder in childhood
- Autosomal Recessive
- The gene for glucose 6 phosphatase is located on chr 11q23
- Muscle not affected because Glucose 6 Phosphate absent in the muscles
- Structure of Glycogen normal.

Biochemical Defect

- Type Ia GSD-Glucose 6 Phosphatase absent or deficient in liver, kidney and intestinal mucosa
- Type Ib GSD-Translocase that transport Glucose 6 Phosphate across endoplasmic reticulum membrane is defective.

Clinical Presentation

Most commonly present at 3–4 months of age with

- Doll like facies with fat cheeks
- Relatively thin extremities
- Short stature, Protuberant abdomen
- Massive Hepatomegaly
- **Kidneys are also enlarged**
- **No Splenomegaly**
- **Plasma may be milky due to associated hypertriglyceridemia.**

Type Ib has additional features of recurrent bacterial infection due to neutropenia and impaired neutrophil dysfunction.

The biochemical hallmarks are

- Hypoglycemia
- Lactic Acidosis
- Hyperlipidemia
- Hyperuricemia

Long term effects of Type I GSD are PCOD, Pancreatitis, Hepatic Adenomas, Pulmonary hypertension, Osteopenia, Renal disease.

Type III GSD (Limit Dextrinosis)

- Autosomal Recessive
- The gene located on chr 1p21.

Biochemical Defect

- Debranching enzyme is defective
- Abnormal glycogen with short outer branch chain resembling limit dextrin accumulate.

Clinical Features

- Hypoglycaemia, Hepatomegaly, hyperlipidemia, short stature, variable skeletal muscle myopathy
- Kidneys are not enlarged
- Splenomegaly may be present
- Progressive liver cirrhosis and failure occurs.

Definite Diagnosis

- Enzyme assay in liver, muscle, or both
- Mutation analysis can provide a noninvasive method for diagnosis and subtype assignment in the majority of patients.

Type IV Glycogen Storage Disease (Amylopectinosis, or Andersen Disease)

- Autosomal recessive
- The glycogen branching enzyme gene is located on chromosome 3p21.

Biochemical Defect

- Deficiency of Branching Enzyme activity results in accumulation of an abnormal glycogen with poor solubility
- The disease is referred to as type IV GSD or amylopectinosis because the abnormal glycogen has a structure resembling amylopectin.

Clinical Manifestations

- This disorder is clinically variable
- The most common and classic form is characterized by progressive cirrhosis of the liver and is manifested in the 1st 18 mo of life as hepatosplenomegaly and failure to thrive
- The cirrhosis progresses to portal hypertension, ascites, esophageal varices, and liver failure that usually leads to death by 5 years of age.

Diagnosis

- Tissue deposition of amylopectin-like materials can be demonstrated in liver, heart, muscle, skin, intestine, brain, spinal cord, and peripheral nerve
- The hepatic histologic findings are characterized by micronodular cirrhosis
- Electron microscopy shows accumulation of the fibrillar aggregations that are typical of amylopectin.

The definitive diagnosis

- Demonstration of the deficient branching enzyme activity in liver, muscle, cultured skin fibroblasts, or leukocytes
- Identification of disease-causing mutations in the glycogen branching enzyme (*GBE*) gene
- Prenatal diagnosis is possible by measuring the enzyme activity in cultured amniocytes, chorionic villi, or mutation analysis.

Muscle Glycogen Storage Disorders

Type	Name	Enzyme defect	Characteristics
II	Pompes Disease (Belongs to lysosomal storage disorder)	Lysosomal α1,4 and α1,6 glucosidase (acid maltase)[Q]	Cardiomegaly, hypotonia, hepatomegaly; cardiorespiratory failure leading to death by age 2 year
	Danon disease	Lysosome-associated membrane protein 2 (LAMP2)	Hypertrophic cardiomyopathy Rare X linked

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Type	Name	Enzyme defect	Characteristics
V	McArdle's syndrome	Muscle phosphorylase[Q]	Poor exercise tolerance muscle glycogen abnormally high
VII	Tarui's disease	Muscle and erythrocyte phosphofructokinase 1	Poor exercise tolerance; Hemolytic anemia myoglobinuria

Pompe Disease (Type II GSD)

Autosomal recessive

Biochemical Defect

- Deficiency of acid α-1, 4-glucosidase (acid maltase), an enzyme responsible for the degradation of glycogen in lysosomes
- The gene for acid α-glucosidase is on chromosome 17q
- Lysosomal glycogen accumulation in multiple tissues and cell types, with cardiac, skeletal, and smooth muscle cells being the most seriously affected.

Clinical Picture

Present in the 1st few months of life

- Hypotonia
- A generalized muscle weakness with a 'floppy infant' appearance
- Feeding difficulties
- Macroglossia
- Hepatomegaly
- Hypertrophic cardiomyopathy
- Followed by death from cardiorespiratory failure or respiratory infection usually by 1 year of age. Juvenile and adult-onset disease (late-onset Pompe disease) is characterized by a lack or absence of severe cardiac involvement and a less severe short-term prognosis.

Laboratory Diagnosis

- Serum creatine kinase, aspartate aminotransferase, lactate dehydrogenase, acid Phospahatase
- Chest x-ray showing massive cardiomegaly
- Electrocardiographic findings include a high-voltage QRS complex and a shortened PR interval. Echocardiography reveals thickening of both ventricles and/or the intraventricular septum and/or left ventricular outflow tract obstruction
- Muscle biopsy shows the presence of vacuoles that stain positively for glycogen

- Electron microscopy reveals glycogen accumulation within the membranous sac and in the cytoplasm. Electromyography reveals myopathic features with excessive electrical irritability of muscle fibers and pseudomyotonic discharges
- Enzyme assayin muscle, cultured skin fibroblasts
- Gene sequencing.

Treatment

Specific enzyme replacement therapy (ERT) with recombinant human acid α-glucosidase (alglucosidase alfa, (Myozyme) is available for treatment of Pompe disease.

Glycogen Storage Diseases Mimicking Hypertrophic Cardiomyopathy

- Lysosomal-associated membrane protein 2 (LAMP2, also called Danon disease)
- AMP-activated protein kinase γ2 (PRKAG2)
  - Both result in accumulation of glycogen in the heart and skeletal muscle.

Type V Glycogen Storage Disease (McArdle Disease)

- Type V GSD is an autosomal recessive disorder.

Biochemical Defect

- Muscle Phosphorylase Defect
- The gene for muscle phosphorylase (PYGM) has been mapped to chromosome 11q13
- Lack of this enzyme limits muscle ATP generation by glycogenolysis, resulting in muscle glycogen accumulation, and is the prototype of muscle energy disorders.

Clinical Manifestations

- Symptoms usually 1st develop in late childhood or as an adult and are characterized by exercise intolerance with muscle cramps and pain
- Many patients experience a characteristic 'second wind' phenomenon. If they slow down or pause briefly at the 1st appearance of muscle pain, they can resume exercise with more ease
- About 50% of patients report burgundy-colored urine after exercise, which is the consequence of exercise-induced **myoglobinuria** secondary to **rhabdomyolysis.**

Diagnosis

- Ischemic exercise test offers a rapid diagnostic screening for patients with a metabolic myopathy
- Lack of an increase in blood lactate levels and exaggerated blood ammonia elevations indicate muscle glycogenosis
- Phosphorus magnetic resonance imaging (^{31}P MRI) allows for the noninvasive evaluation of muscle metabolism
- Enzyme Studies.

Treatment

- Avoidance of strenuous exercise
- Glucose or sucrose given before exercise or injection of glucagon can markedly improve tolerance in these patients
- Vitamin B6 supplementation reduces exercise intolerance and muscle cramps.

Type VII Glycogen Storage Disease (Tarui Disease)

- Autosomal recessive disorder
- The gene for muscle phosphofructokinase is located on chromosome 12q13.3.

Biochemical Defect

Deficiency of **muscle phosphofructokinase (M Isoenzyme form)**, which catalyzes the ATP-dependent conversion of fructose-6-phosphate to fructose-1,6-diphosphate.

Isoforms of PFK and defect in Tarui Disease

- Phosphofructokinase is composed of 3 isoenzyme subunits (M [muscle], L [liver], and P [platelet]) that are encoded by different genes and differentially expressed in tissues
- Skeletal muscle contains only the M subunit, and red blood cells contain a hybrid of L and M forms
- Type VII disease is due to a defective M isoenzyme, which causes a complete enzyme defect in muscle and a partial defect in red blood cells.

Clinical Manifestations

- Exercise intolerance
- Compensated hemolysis
- Hyperuricemia is common and exaggerated by muscle exercise
- An abnormal polysaccharide is present in muscle fibers; it is periodic acid–Schiff positive but resistant to diastase digestion
- Exercise intolerance is particularly acute after meals that are rich in carbohydrates because glucose cannot be utilized in muscle and because glucose inhibits

lipolysis and thus deprives muscle of fatty acid and ketone substrates
- There is no spontaneous second-wind phenomenon because of the inability to metabolize blood glucose.

Diagnosis
- Enzymatic defect demonstrated in muscle
- The absence of the M isoenzyme of phosphofructokinase can also be demonstrated in blood cells and fibroblasts.

Treatment
- There is no specific treatment
- Avoidance of strenuous exercise is advisable to prevent acute attacks of muscle cramps and myoglobinuria.

Glycogen Storage Disorders (GSDs) at a glance
- Most common GSD in adolescent and adults: Type V GSD (McArdles Disease)
- Liver GSD disorder causes fasting hypoglycemia and hepatomegaly
- GSDs associated with liver cirrhosis: Type III, Type IV, Type IX GSDs
- GSD associated with renal dysfunction: Type I GSD
- Liver GSD with myopathy: Type III GSD and Type IV GSD
- Liver GSD with neurological (brain and anterior horn cells) involvement: Type II GSD

MINOR METABOLIC PATHWAYS
- HMP Shunt Pathway
- **Uronic Acid Pathway**
- **Polyol Pathway**
- **Galactose Metabolism**
- **Fructose Metabolism.**

HMP Shunt Pathway
- **Other names:** Pentose Phosphate Pathway, Dicken Horecker Pathway, Phosphogluconate Pathway
- Organelle-Cytosol
- Rate limiting step-Glucose 6 Phosphate Dehydrogenase.

Biochemical Significances of HMP Pathway
- Alternative route for metabolism of Glucose
- Complete oxidation of Glucose
- More complex pathway than Glycolysis
- Major function is to generate NADPH and Riboses
- No ATP is generated by this pathway. NBE Pattern QAI-08)

HMP Shunt Pathway has Two Phases

Three molecules of Glucose 6 phosphate give rise to three molecules of CO_2 and three 5 Carbon sugars. They are rearranged to regenerate two molecules of Glucose 6 Phosphate and one molecule of Glyceraldehyde 3 Phosphate. This is taking place in two phases.

1. Oxidative Phase
2. Nonoxidative Phase

Characteristics of the two phases of HMP shunt pathway

Oxidative Phase—Characteristics and Organs

Sites: Liver, Adipose Tissue, Adrenal Cortex, Erythrocytes, Gonads, Thyroid, Lactating Mammary Glands (Not in non lactating mammary glands)
- Irreversible
- Takes place in sites where NADPH is required
- Glucose 6 Phosphate undergoes dehydrogenation and decarboxylation to Ribulose 5 Phosphate
- Oxidation is achieved by dehydrogenation using NADP+, not NAD+, as the hydrogen acceptor
- Produce NADPH(TNPGEE 2008) DNB 2012

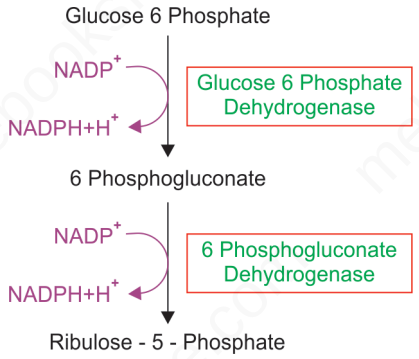


Fig. 5.17: Oxidative phase of HMP pathway

Uses of NADPH
- Used to prevent oxidative Damage—RBC, Lens by keeping glutathione in the reduced state
- Reductive biosynthesis of fatty acids and Steroids.

Nonoxidative Phase regenerate Glucose 6 Phosphate

Sites: In rapidly dividing cells bone marrow, skin, intestinal mucosa and virtually in all tissues.
- Reversible
- Produce Pentoses
- Ribulose 5-phosphate is converted back to glucose 6-phosphate mainly by two transketolase reaction and one transaldolase.

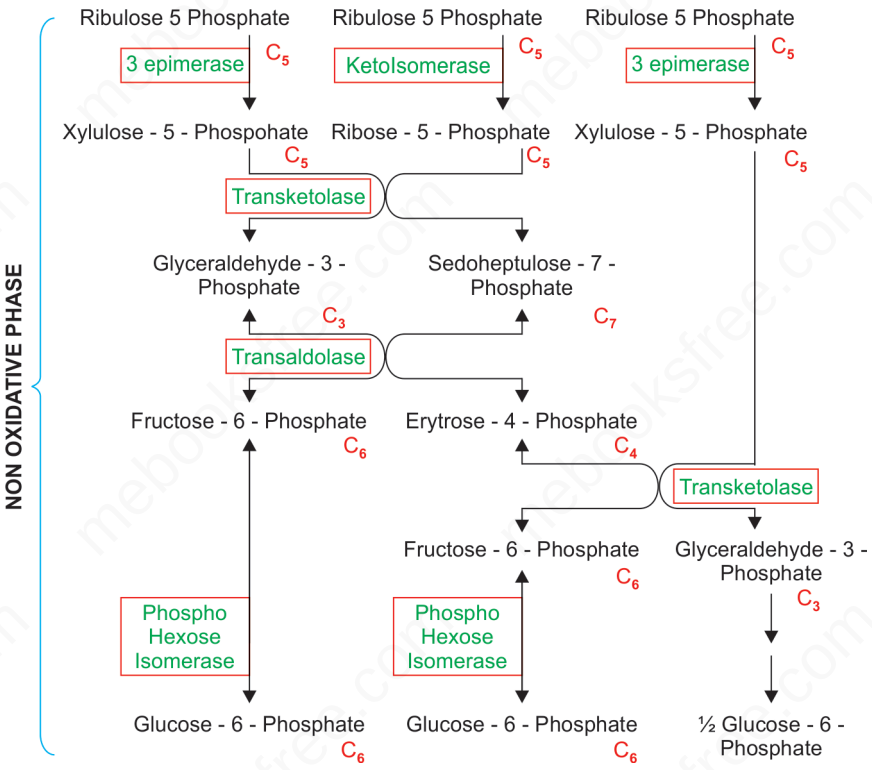


Fig. 5.18: Nonoxidative phase of HMP pathway

Transketolase

Two transketolase reactions

- Transfers the two-carbon unit comprising carbons 1 and 2 of a ketose onto the aldehyde carbon of an aldose sugar
- It therefore affects the conversion of a ketose sugar into an aldose with two carbons less and an aldose sugar into a ketose with two carbons more
- Require thiamine as coenzyme
- Erythrocyte transketolase[Q] is a measure thiamine status of the body.

Transaldolase

One transaldolase reaction

Transfer the three carbon unit of a 7 carbon keto sugar (sedoheptulose 7 phosphate) to a 3 C aldosugar (Glyceraldehyde 3 Phosphate) to form a 6 carbon Keto Sugar (Fructose 6 Phosphate) and 4 carbon aldo Sugar.

No cofactor for this enzyme.

Metabolites in HMP Shunt Pathway

- Glucose 6 Phosphate
- 6 Phosphogluconate
- Ribulose 5 Phosphate
- Xylulose 5 Phosphate

Contd...

Contd...

- Ribose 5 Phosphate
- Glyceraldehyde 3 Phosphate
- Sedoheptulose 7 Phosphate
- Fructose 6 phosphate
- Erythrose 4 Phosphate

Key points to remember in HMP (Pentose Phosphate) Pathway

- Main source of NADPH[Q2012] and Pentoses.
- Two transketolation and one transaldolation reactions are involved.
- No ATP is produced[Q]
- CO_2 is produced in this pathway and not in glycolytic Pathway[Q] DNB 01)
- Deficiency of Glucose 6 phosphate dehydrogenase is a major cause of acute hemolysis in erythrocytes
- NADPH is used for **reductive biosynthetic pathways,** like fatty acid synthesis, steroid synthesis, Amino acids by Glutamate dehydrogenase
- NADPH is required for regeneration of reduced Glutathione, that clears free radicals from erythrocytes and lens.

Clinical Correlation—HMP Pathway

Glucose 6 Phosphate Dehydrogenase Deficiency

Most common enzyme deficiency in human beings.

X-linked recessive

Manifest as

- Hemolytic Anemia
- Methemoglobinemia

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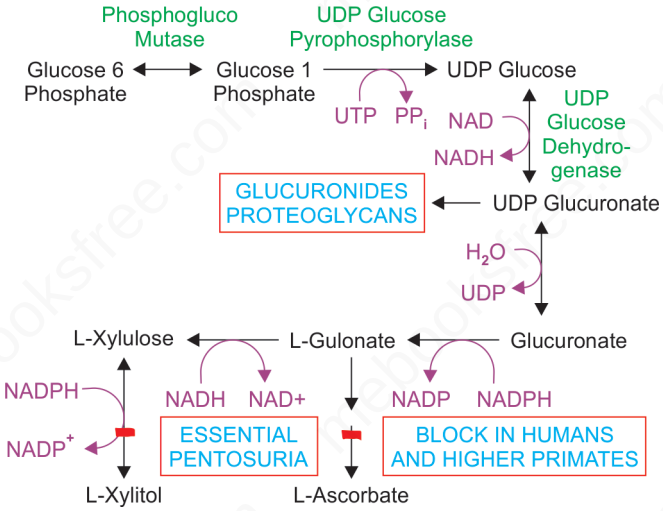
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Due to decrease in NADPH which clears the free radicals in the RBCs and keep the iron in the hemoglobin in the reduced state. These people are sensitive to certain drugs like

- Primaquine, Aspirin, Sulfadrugs
- Consumption of favabeans (vicia fava) can also precipitate hemolysis. (Favism)

Uronic Acid Pathway

- An alternative oxidative pathway for glucose
- UDP Glucose converted to UDP Glucuronic Acid (Glucuronate) by using NAD+ (DNB 93) (TNPGEE 89)
- No ATP is formed
- *Site-liver*
- *Organelle-cytosol*



Biochemical Role

- **To Produce Glucuronic Acid**
  - Source of Glucuronate for the synthesis of Glycosaminoglycans and Proteoglycans
  - For Phase II Conjugation reaction (Conjugation of Bilirubin and many drugs).
- **To produce Ascorbic Acid**
  - But not in higher primates and mammals
  - Because they lack **L-Gulonolactone Oxidase**Q
- **To Produce Pentoses**

Clinical Correlation: Uronic Acid Pathway

Essential Pentosuria a defect in Uronic Acid PathwayQ

- Benign condition
- One among the Garrod's tetrad (Pentosuria, Albinism, Cystinuria, Alkaptonuria)
- Due to deficiency of Xylitol Dehydrogenase Q 2012
- L-Xylulose excreted in urine
- Gives Benedict's Test Positive
- Bial's Test Positive.

Various drugs increase the rate at which glucose enters the uronic acid pathway.

For example, administration of barbital or chlorobutanol to rats results in a significant increase in the conversion of glucose to glucuronate, L-gulonate, and ascorbate.

Aminopyrine and antipyrine increase the excretion of L-xylulose in pentosuric subjects.

Pentosuria also occurs after consumption of relatively large amounts of fruits such as pears that are rich sources of pentoses **(alimentary pentosuria).**

Polyol/Sorbitol Pathway

- To produce Fructose from Glucose
- Responsible for occurrence of Fructose in seminal fluid
- May be responsible for the pathogenesis of Diabetic Cataract
- The pathway is increasingly seen as glucose concentration rises in those tissues which are not insulin sensitive, like the lens, peripheral nerves, renal glomeruli
- Glucose is reduced to Sorbitol by Aldose Reductase.
- Sorbitol is oxidized to Fructose by Sorbitol dehydrogenase
- Sorbitol is responsible for Diabetic cataract because, it cannot pass through cell membrane so accumulate, causing osmotic damage.

Metabolism of Galactose

Galactose is seen in:

- Lactose
- Glycolipids
- Glycoproteins
- GAG (Keratan Sulfate) (TNPGEE 2010)

Galactose is derived from intestinal hydrolysis of the disaccharide **lactose,** the sugar of milk. It is readily converted in the liver to glucose.

Conversion of Galactose to Glucose

Rate limiting Step-**Galactose 1 Phosphate uridyl Transferase**

Site: Galactose is metabolized exclusively in the Liver, hence Galactose Tolerance Test is a Liver Function Test.Q

- Galactokinase catalyzes the phosphorylation of galactose, using ATP as phosphate donor

- Galactose 1-phosphate reacts with UDPGlc to form uridine diphosphate galactose (UDPGal) and glucose 1-phosphate, in a reaction catalyzed by galactose 1-phosphate uridyl transferase
- The conversion of UDPGal to UDPGlc is catalyzed by UDPGal 4-epimerase
- The UDPGlc is then incorporated into glycogen
- In the synthesis of lactose in the mammary gland, UDPGal condenses with glucose to yield lactose, catalyzed by lactose synthase.

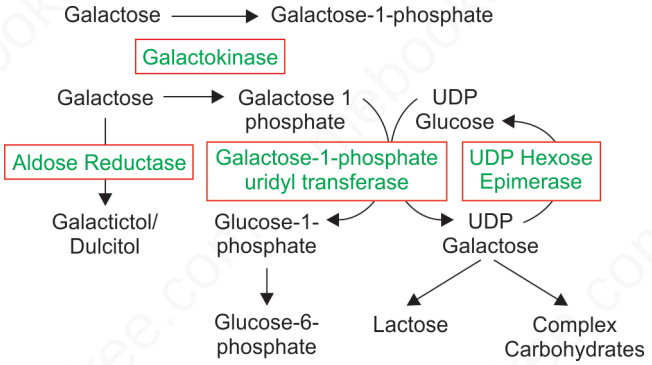


Fig. 5.19: Metabolism of galactose

Galactosemia (Very Important Topic)

Enzyme deficiency (AIIMS Nov 2011, May 2013)

- Galactose-1-phosphate uridyltransferase [Classic Galactosemia]
- Galactokinase
- UDP Hexose 4 Epimerase.

The newborn infant receives high amounts of lactose, which consists of equal parts of glucose and galactose. Without the galactose-1-phosphate uridyltrasferase enzyme, the infant is unable to metabolize galactose-1-phosphate, the accumulation of which results in injury to kidney, liver, and brain.

Clinical features

- Classic galactosemia is a serious disease with onset of symptoms typically by the 2nd half of the 1st week of life
- With jaundice, vomiting, seizures, lethargy, irritability, feeding difficulties, poor weight gain or failure to regain birth weight
- Hepatomegaly Oil drop cataracts, Hepatic failure
- Mental retardation
- Patients with galactosemia are at increased risk for Escherichia coli neonatal sepsis.

Diagnosis

- Demonstrating a reducing substance in several urine specimens collected while the patient is receiving human milk, cow's milk, or any other formula containing lactose
- Benedict's test positive^{Q}
- Chromatography for presence of Galactose in urine.
- Clinistix urine test results are usually negative because the test materials rely on the action of glucose oxidase, which is specific for glucose and is nonreactive with galactose (Glucose Oxidase Test is negative)
- Mucic Acid Test Positive
- Galactose Tolerance Test is contraindicated
- Direct enzyme assay using erythrocytes establishes the diagnosis.

Treatment

- Lactose free diet till 4-5 years of age
- Because galactose-1-phosphate pyrophosphorylase becomes active by 4–5 years. This enzyme reduces the level of galactose-1-phosphate
- Breast milk is avoided^{Q}

Metabolism of Fructose

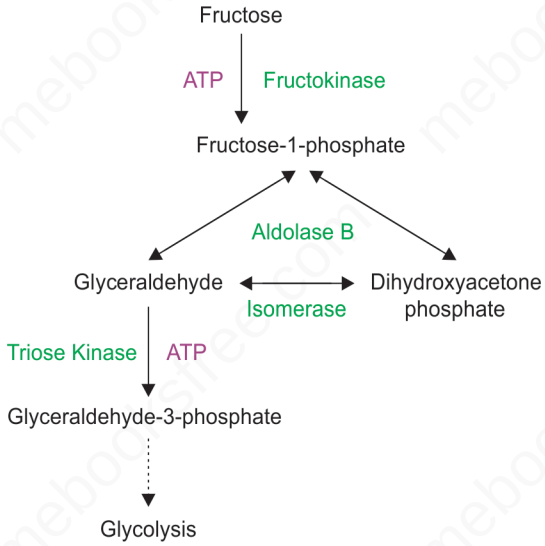


Fig. 5.20: Metabolism of fructose

Key Points in the Metabolism of Fructose

Fructose undergoes more rapid glycolysis in the liver than does glucose, because it bypasses the regulatory step catalyzed by phosphofructokinase.

This allows fructose to flood the pathways in the liver, leading to enhanced fatty acid synthesis, increased

esterification of fatty acids, and increased VLDL secretion, which may raise serum triacylglycerols and ultimately raise LDL cholesterol concentrations.

Fructokinase, in liver, kidney, and intestine, catalyzes the phosphorylation of fructose to fructose-1-phosphate. Unlike glucokinase, its activity is not affected by fasting or by insulin, which may explain why fructose is cleared from the blood of diabetic patients at a normal rate

Fructose-1-phosphate is cleaved to D-glyceraldehyde and dihydroxyacetone phosphate by aldolase B.

Clinical Correlations

Hereditary fructose intolerance

Autosomal Recessive

Biochemical defect

- Deficiency of fructose-1,6-bisphosphate Aldolase (Aldolase B)
- The gene for aldolase B is on chromosome 9q22.3
- Deficiency of this enzyme activity causes a rapid accumulation of fructose-1-phosphate and initiates severe toxic symptoms when exposed to fructose.

Clinical manifestations

- Patients with HFI are asymptomatic until fructose or sucrose (table sugar) is ingested (usually from fruit, fruit juice, or sweetened cereal)
- Symptoms may occur early in life, soon after birth if foods or formulas containing these sugars are introduced into the diet
- Early clinical manifestations resemble galactosemia and include jaundice, hepatomegaly, vomiting, lethargy, irritability, and convulsions, hypoglycemia
- Acute fructose ingestion produces symptomatic hypoglycemia. If the intake of the fructose persists, hypoglycemic episodes recur, and liver and kidney failure progress, eventually leading to death
- Chronic ingestion results in failure to thrive.

Laboratory diagnosis

- Prolonged clotting time, hypoalbuminemia, elevation of bilirubin and transaminase levels
- Proximal tubular dysfunction
- Definitive diagnosis is made by assay of fructaldolase B activity in the liver
- Gene-based diagnosis for the mutation.

Treatment

Complete exclusion of all sources of fructose.

Essential Fructosuria (Benign Fructosuria)

- Autosomal recessive. Benign condition
- It is an accidental finding usually made because the asymptomatic patient's urine contains a reducing substance.

Biochemical defect

- Fructokinase defect
- The gene encoding fructokinase is located on chromosome 2p23.3
- Fructokinase catalyzes the 1st step of metabolism of dietary fructose: conversion of fructose to fructose-1-phosphate
- Without this enzyme, ingested fructose is not metabolized. Its level is increased in the blood, and it is excreted in urine because there is practically no renal threshold for fructose.

Lab diagnosis

- Rapid furfural test and Seliwanoff's test positive
- Clinistix for reducing sugars
- Urine chromatography for fructose.

Loading of the Liver with Fructose may Potentiate Hypertriacylglycerolemia, Hypercholesterolemia, and Hyperuricemia

- In the liver, fructose increases fatty acid and triacylglycerol synthesis and VLDL secretion, leading to hypertriacylglycerolemia—and increased LDL cholesterol—which can be regarded as potentially atherogenic.
- Acute loading of the liver with fructose, as can occur with intravenous infusion or following very high fructose intakes, causes sequestration of inorganic phosphate in fructose-1-phosphate and diminished.

METABOLISM OF CARBOHYDRATES

ATP synthesis. As a result, there is less inhibition of de novo purine synthesis by ATP, and uric acid formation is increased, causing hyperuricemia, which is the cause of gout.

Since fructose is absorbed from the small intestine by (passive) carrier-mediated diffusion, high oral doses may lead to osmotic diarrhea.

Fructose and Sorbitol in the Lens Are Associated with Diabetic Cataract

- Both fructose and sorbitol are found in the lens of the eye in increased concentrations in diabetes mellitus and may be involved in the pathogenesis of **diabetic cataract**. The **sorbitol (polyol) pathway** (not found in liver) is responsible for fructose formation from glucose
- The glucose concentration rises in those tissues that are not insulin-sensitive, i.e. the lens, peripheral nerves, and renal glomeruli
- This increases activity of Sorbitol pathway

Contd...

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- Glucose is reduced to sorbitol by **aldose reductase,** followed by oxidation of sorbitol to fructose in the presence of NAD+ and sorbitol dehydrogenase (polyol dehydrogenase)
- Sorbitol does not diffuse through cell membranes, but accumulates, causing osmotic damage. Simultaneously, myoinositol levels fall
- In experimental animals, sorbitol accumulation and myoinositol depletion, as well as diabetic cataract, can be prevented by aldose reductase inhibitors.

INTEGRATION OF METABOLISM

Major Metabolic Fuel of Major Organs[Q]

Organ	Major metabolic fuels
Liver	Free fatty acids, glucose (in fed state), lactate, glycerol, fructose, amino acids, alcohol
Brain	Glucose, amino acids, ketone bodies in prolonged starvation
Heart[Q]	Ketone bodies, free fatty acids, lactate, chylomicron and VLDL triacylglycerol, some glucose
Adipose tissue	Glucose, chylomicron and VLDL triacylglycerol
Fast Twitch Muscle	Glucose, glycogen
Slow Twitch Muscle	Ketone bodies, chylomicron and VLDL triacylglycerol
Kidney	Free fatty acids, lactate, glycerol, glucose
Erythrocyte	Glucose

STARVE FEED CYCLE

Divided into 5 stages:

- Well-fed state (1–4 hours after food)
- Early fasting (4–16 hours after food)
- Fasting (16–48 hours after food)
- Starvation (2–3 days without food)
- Prolonged starvation (> 5 days without food).

Stage I Well-Fed State

- Exogenous dietary supply of Fuel from the Intestine
- Glucose is the major fuel
- Insulin is the hormone: Most of the enzyme regulated by covalent modification is in the Dephosphorylated State
- Favor Glucose utilization and storage of excess glucose.[Q]

In the Liver

On Carbohydrate Metabolism

- Favor Glycolysis
- Favor Glycogenesis
- Uptake of Glucose by GLUT-2 is insulin independent But Glucokinase is an inducible enzyme by insulin, when Glucose is excess
- Decreased Gluconeogenesis.

Fat metabolism

- Favor Lipogenesis[Q]
- Increased Fatty Acid Synthesis
- Increased Triacyl Glycerol Synthesis.

In adipose tissue

- Favor the transport of GLUT-4 from intracellular vesicle to the cell surface[Q]
- Favor the uptake of glucose
- Inhibit hormone sensitive Lipase[Q]-Inhibit Lipolysis Favor
- Fatty acid synthesis. Favor Triacylglycerol Synthesis.

In the muscle

- Glucose uptake by insulin dependent GLUT-4
- Favor Glycolysis
- Favor Glycogenesis.

Post-absorptive Phases

No fuel from gut. Plasma insulin Decreases, Glucagon level begin to rise. Increased cAMP level–Increased cAMP dependent Protein Kinase A.

Most of the enzyme active in postabsorptive phase are in Phosphorylated state.

Glucagon

- Activate Adenylyl Cyclase
- Increase cAMP level.

Insulin

- Favor Phosphodiesterase which convert cAMP to 5′AMP
- Decrease cAMP level.[(DNB 2011, AIIMS May 95)]

Stage II Early Fasting (4–16 Hours after Food)

Fuel from the gut stopped

On Carbohydrate metabolism

- Increased break down of liver glycogen (not Muscle)
- Decreased Glycogenesis.

On Lipid Metabolism

Decreased Lipogenesis

Stage III Fasting (16–48 Hours After Food)

- No fuel from the gut
- By 12–18 hours liver Glycogen[Q] stores are depleted

- Hepatic Gluconeogenesis favored
- Muscle Proteins are degraded to supply amino group to Pyruvate to form Alanine
- This Alanine reaches the liver used for gluconeogenesis. This is Glucose Alanine Cycle.

Stage IV Starvation or Prolonged Fasting (2–4 Days Without Food)

- No fuel from the gut
- Decreased Gluconeogenesis
- Increased activity of hormone sensitivity lipase
- Increased lipolysis, Free Fatty Acid level rises
- Increased Ketone Bodies synthesis
- Ketone Bodies inhibit Muscle proteolysis.

Stage V Prolonged Starvation (after 5 days)

Increased Proteolysis: Muscle Wasting Cachexia

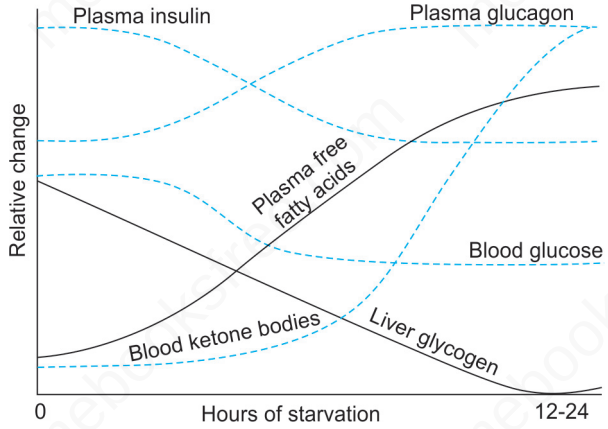


Fig. 5.21: Biochemical changes in fasting

REVIEW QUESTIONS

Glycolysis

1. Irreversible steps of Glycolysis are catalyzed by: (AIIMS May 2013)

a. Hexokinase, Phosphofructokinase, Pyruvate Kinase
b. Glucokinase, Pyruvate Kinase, Glyceraldehyde 3 Phosphate Dehydrogenase
c. Hexokinase, Phospho Glycerate Kinase, Pyruvate Kinase
d. Pyruvate Kinase, Fructose 1,6 Bisphosphatase, Phosphofructokinase

Ans. a. Hexokinase, Phosphofructokinase, Pyruvate Kinase *(Ref: Harper 29/e p171-174, 30/e p170, 172)*

Remember
All the Kinases are irreversible except 1,3 Bisphosphoglycerate Kinase which is reversible.

2. Glycolysis occurs in: (AIIMS May 2007)

a. Cytosol
b. Mitochondria
c. Nucleus
d. Lysosome

Ans. a. Cytosol *(Ref: Harper 29/e p171, 30/e p170)*

Metabolic	Pathway Site
Glycolysis	Cytoplasm
Gluconeogenesis	Cytoplasm and Mitochondria
Glycogen Synthesis	Cytoplasm

Contd...

Contd...

Metabolic	Pathway Site
Glycogenolysis	Cytoplasm and some in Lysosomes
HMP Pathway	Cytoplasm
Pyruvate Dehydrogenase	Mitochondria
Krebs Cycle	Mitochondria

3. Irreversible step(s) in Glycolysis is/are: (PGI May 2012)

a. Enolase
b. Phosphofructokinase
c. Pyruvate Kinase
d. Glyceraldehyde 3 Phosphate Dehydrogenase
e. Hexokinase

Ans. b, c and e. *(Ref: Harper 29/e p171, 172, 30/e p170-172)*

Irreversible Steps of Glycolysis[Q]

- Hexokinase
- Phosphofructokinase
- Pyruvate Kinase.

Remember
All the Kinases are irreversible except 1,3 Bisphospho Glycerate Kinase which is reversible.

Substrate Level Phosphorylation[Q]

- Phosphoglycerate Kinase (1,3 Bisphosphoglycerate to 3 Phosphoglycerate)
- Pyruvate Kinase (Phosphoenolpyruvate to Pyruvate).

NB: Learn the enzyme and the reaction. Question can be asked in either ways.

4. Enzyme catalyzing reversible step in glycolysis is: (PGI Nov 2010)

a. Phosphofructokinase
b. Enolase
c. Pyruvate kinase
d. Phosphoglycerate mutase
e. Glyceraldehyde-3-Phosphate Dehydrogenase

Ans. b, d and e. *(Ref: Harper p171, 172, 30/e p170)*

5. In which of the following steps ATP is released? (Kerala 2008)

a. Phosphoenol pyruvate to pyruvate
b. Glyceraldehyde 3 phosphate to 1,3 bisphosphoglycerate
c. Fructose 6 phosphate to fructose 1,6 bisphosphate
d. Glucose to Glucose 6 phosphate

Ans. a. Phosphoenol pyruvate to pyruvate

(Ref: Harper 30/e p170, 172)

Steps releasing ATP at the level of substrate

- 1, 3 Bisphosphoglycerate to 3 Phosphoglycerate (1, 3 Bisphosphoglycerate Kinase)
- Phosphoenolpyruvate to Pyruvate. (Pyruvate Kinase)
- Succinyl CoA to Succinate (Succinate Thiokinase)

6. What activate Kinases of glycolysis? (NBE Pattern Q)

a. ATP
b. c AMP
c. Insulin
d. Glucagon

Ans. c. Insulin *(Ref: Harper 30/e p188 Table 19-1)*

Regulation of Carbohydrate Metabolism
(NB: This table is an important topic for all exams)

Enzyme	Inducer	Repressor	Activator	Inhibitor
Glycogen synthase	Insulin	Glucagon	Insulin, glucose 6-phosphate	Glucagon
Hexokinase		Glucagon		Glucose 6-phosphate
Glucokinase	Insulin	Glucagon		
Phosphofructokinase-1	Insulin	**Glucagon[Q]**	**5'AMP, fructose 6-phosphate, fructose 2, 6-bisphosphate[Q] Inorganic Phosphate**	**Citrate, ATP, glucagon**

Contd...

Contd...

Enzyme	Inducer	Repressor	Activator	Inhibitor
Pyruvate kinase	***Insulin***	**Glucagon[Q]**	**Fructose 1,6-bisphosphate, insulin**	**ATP alanine glucagon norepinephrine**
Pyruvate dehydrogenase	***Insulin***	**Glucagon[Q]**	**CoA, NAD+, insulin[Q], ADP, pyruvate**	**Acetyl CoA[Q], NADH, ATP (fatty acids, ketone bodies)**

Gluconeogenesis				
Enzyme	**Inducer**	**Repressor**	**Activator**	**Inhibitor**
Pyruvate carboxylase	Glucocorticoids, Glucagon, Epinephrine	Insulin	Acetyl CoA[Q]	ADP
Phosphoenolpyruvate carboxykinase	Glucocorticoids, Glucagon, Epinephrine	Insulin		
Glucose 6-phosphatase	Glucocorticoids, Glucagon, Epinephrine	Insulin		

7. True statement about glycolysis is: (PGI Dec 98)

a. Occurs in mitochondria
b. Complete break down of glucose
c. Conversion of glucose to 3C units
d. 3 ATPs are used in anaerobic pathway

Ans. c. Conversion of Glucose to 3 C units

(Ref: Harper 30/e p171)

Option a. Glycolysis occur in cytosol
Option b. Complete break down of Glucose happens when Pyruvate formed by Glycolysis undergo Pyruvate Dehydrogenase reaction, followed by TCA Cycle.
Option d. In anaerobic Glycolysis

- **Number of ATPs produced is 4**
- **Number of ATPs used is 2**
- **Net ATP yield by anaerobic glycolysis is 2.**

8. Compound that joins glycolysis with glycogenesis and glycogenolysis: (JIPMER 04)

a. Glucose 1,6 bisphosphate
b. Glucose 1 PO_4

c. Glucose 6 PO_4
d. Fructose 1,6 bisphosphate

Ans. c. Glucose 6 Phosphate *(Ref: Harper 30/e p170)*

Fates of Glucose 6 Phosphate

- Can undergo Glycolysis
- Can enter into Glycogenesis
- Can be used for gluconeogenesis
- Is an intermediate in Glycogenolysis
- Can enter in to HMP Pathway.

9. Key glycolytic enzymes: (PGI 98)

a. Phosphofructokinase
b. Hexokinase
c. Pyruvate kinase
d. Glucose 1, 6 bisphosphatase

Ans. a, b and c. *(Ref: Harper 30/e p170)*

Irreversible steps of Glycolysis are:

- Hexokinase/Glucokinase
- Phosphofructokinase
- Pyruvate Kinase.

10. In glycolysis the first committed step is catalyzed by: (AIIMS Dec 97)

a. 2,3-DPG
b. Glucokinase
c. Hexokinase
d. Phosphofructokinase

Ans. d. Phosphofructokinase *(Ref: Harper 30/e p173)*

- First Committed step is catalyzed by Phosphofructokinase
- This is otherwise called the bottle neck of Glycolysis.

Hexokinase/Glucokinase

- Convert Glucose to Glucose 6 Phosphate
- Glucose 6 Phosphate has different fates, not only Glycolysis.

Fates of Glucose 6 Phosphate

- Can undergo Glycolysis
- Can enter into Glycogenesis
- Can be used for gluconeogenesis
- Is an intermediate in Glycogenolysis
- Can enter in to HMP Pathway.

11. The rate-limiting enzyme in glycolysis is: (AI 2000)

a. Phosphofructokinase
b. Glucose 6-dehydrogenase
c. Glucokinase
d. Pyruvate kinase

Ans. c. Phosphofructokinase *(Ref: Harper 30/e p170, 188)*

Regulatory steps of Glycolysis are

- Hexokinase/Glucokinase
- Phosphofructokinase
- Pyruvate Kinase.

Harper says Phosphofructokinase occupy a key position in regulating Glycolysis and is also subject to feed back control.

12. Cancer cells derive nutrition from:

a. Glycolysis **(AIIMS Nov 2001)**
b. Oxidative phosphorylation
c. Increase in mitochondria
d. From a fast food joint

Ans. a. Glycolysis *(Ref: Harper 30/e p738)*

In 1924, the biochemist Otto Warburg and his colleagues made the discovery that cancer cells take up large amounts of glucose and metabolize it to lactic acid, even in the presence of oxygen. This observation was termed the **Warburg effect**. Based on these data, Warburg made two hypotheses: first, that the **increased ratio of glycolysis** to aerobic respiration was likely due to defects in the mitochondrial respiratory chain; and second, that this enhanced glycolysis enabled cancer cells to preferentially proliferate in the reduced oxygen tension often seen in tumors. Furthermore, Warburg argued that the switch from aerobic to anaerobic glucose **metabolism was the driver of tumorigenesis.**

13. True statements about glucokinase is/are: (PGI Dec 2003)

a. Km value is higher than normal blood sugar
b. Found in liver
c. Glucose 6 phosphate inhibit it
d. Has both glucose 6 phosphatase and kinase activity
e. Glucose enter into cells through GLUT-2

Ans. a, b and e. *(Ref: Harper 30/e p170, 191)*

- **Glucokinase is important in regulating blood glucose after a meal**
- Glucokinase has a considerably higher Km (lower affinity) for glucose, so that its activity increases
- With increases in the concentration of glucose in the hepatic portal vein
- Found in liver cells and Pancreatic Beta islet cells
- Glucose 6 Phosphate inhibit Hexokinase not Glucokinase
- Glucose enter into liver cells and Pancreatic beta cells through GLUT2.

14. Within the RBC, hypoxia stimulates glycolysis by which of the following regulating pathways: (AI 2007)

a. Hypoxia stimulates pyruvate dehydrogenase by increased 2,3-DPG
b. Hypoxia inhibits hexokinase
c. Hypoxia stimulates release of all glycolytic enzymes from Band 3 on RBC membrane
d. Activation of the regulatory enzymes by high pH

Ans. c. Hypoxia stimulates release of all glycolytic enzymes from Band 3 on RBC membrane

15. All except occurs on decrease in blood glucose level: (AI 2012)

a. Inhibition of PFK-II
b. Activation of Fructose 2,6 Bisphosphatase
c. Increase in glucagon
d. Increase in Fructose 2,6 Bisphosphate

Ans. d. Increase in Fructose 2, 6 Bisphosphate *(Ref: Harper 30/e p191)*

- **On Decreasing Blood Glucose Level**
- ***Glucagon is released from β cells of Pancreas***
- **Increases the Blood Glucose Level by**
- **Inhibiting Glycolysis**
  - By phosphorylating the key enzymes of Glycolysis
  - By decreasing the level of Fructose 2, 6 Bisphosphate, the product of PFK-II
- **Favor Gluconeogenesis**
  - By phosphorylating Key enzymes of Gluconeogenesis
  - By favoring Fructose 2, 6 Bisphosphatase, which decreases the level of Fructose 2, 6 Bisphosphate, a potent activator of PFK-I.

16. The number of ATPs produced by Rapaport leubering Cycle in RBC from Glucose? (NBE pattern Qn)

a. 1
b. 2
c. 3
d. 4

Ans. b. 2 *(Ref: Harper 30/e p173, 174)*

- Rapaport Leubering cycle (2,3 BPG Cycle) takes place in the erythrocytes
- The reaction catalyzed by phosphoglycerate kinase may be bypassed
- 1,3-bisphosphoglycerate is converted to 2,3-bisphosphoglycerate by bisphosphoglycerate 2,3- bisphosphoglycerate
- 2,3 Bisphospho Glycerate is hydrolyzed to 3-phosphoglycerate and Pi by 2,3-bisphosphoglycerate phosphatase mutase
- No ATP is generated by this step
- But 2 ATPs are generated by Pyruvate Kinase
- As 2 ATPs are utilized by Hexokinase and PFK-1, No net ATPs are generated by this pathway.

17. Enzyme responsible for complete oxidation of glucose to CO_2 and water is present in: (AIIMS May 2007)

a. Cytosol
b. Mitochondria
c. Lysosomes
d. Endoplasmic reticulum

Ans. b. Mitochondria *(Ref: Harper 29/e p173, 30/e p172)*

- Under aerobic conditions, pyruvate is taken up into mitochondria, and after oxidative decarboxylation to acetyl-CoA is oxidized to CO_2 by the citric acid cycle
- Under anaerobic conditions, pyruvate is reduced by the NADH to lactate, catalyzed by lactate dehydrogenase.

18. Substrate level phosphorylation is by: (NBE Pattern Q)

a. Pyruvate kinase
b. Phosphofructokinase
c. Hexokinase
d. ATP Synthase

Ans. a. Pyruvate Kinase. *(Ref: Harper 30e p171)*

19. The enzyme not involved in substrate level phosphorylation: (JIPMER 2014)

a. Pyruvate kinase
b. Phosphofructokinase
c. Succinate thiokinase
d. Phosphoglycerate kinase

Ans. b. Phosphofructokinase. *(Ref: Harper 30/e p169)*

Gluconeogenesis

20. Which of the following does not contribute to glucose by gluconeogenesis? (AIIMS Nov 2015)

a. Lactate
b. Acetyl CoA
c. Pyruvate
d. Oxaloacetate

Ans. b. Acetyl CoA *(Ref: Harper 30/e p185, 186)*

Lactate from muscle and RBC are converted to glucose in the liver (Coris Cycle) Lactate and Alanine is converted to Pyruvate which can enter into Gluconeogenesis.

Oxaloacetate is converted to Phosphoenolpyruvate by PEPCK and enter into Gluconeogenesis.

21. True about gluconeogenesis is: (PGI May 2013)

a. Prevent hypoglycemia during prolonged fasting
b. Occur both in muscle and liver
c. Fructose 2, 6 Bisphosphate stimulate it
d. Excess acetyl CoA causes stimulation
e. Carbon skeleton of amino acids are involved in gluconeogenesis

Ans. a, d and e.

- Gluconeogenesis occur in liver and Kidney
- Fructose 2, 6 Bisphosphate activate PFK-I, hence stimulate Glycolysis.

22. A child with low blood glucose is unable to do glycogenolysis or gluconeogenesis. Which of the following enzyme is missing in the child? (AIIMS Nov 2012)

a. Fructokinase
b. Glucokinase
c. Glucose 6 Phosphatase
d. Transketolase

Ans. c. Glucose 6 Phosphatase

(Ref: Harper 29/e p179, 181 Table 19.2, 189; 30/e p177)

The answer should be an enzyme common to Glycogenolysis and Gluconeogenesis.

23. In fasted state gluconeogenesis is promoted by which enzyme: (AIIMS May 2012)

a. Acetyl CoA induced stimulation of Pyruvate Carboxylase
b. Citrate induced stimulation of Acetyl CoA Decarboxylase
c. Fructose 2,6 bisphosphate induced stimulation of Phosphofructokinase-1
d. Stimulation of Pyruvate kinase by Fructose 1,6 Bisphosphate

Ans. a. Acetyl CoA induced stimulation of Pyruvate Carboxylase

(Ref: Harper 29/e p190 Table 20-1; 30/e p188 Table 19.1)

Regulation of Carbohydrate Metabolism (NB: This table is an important topic for all exams)

Enzyme	Inducer	Repressor	Activator	Inhibitor
Glycogen synthase	Insulin	Glucagon	Insulin, glucose 6-phosphate	Glucagon
Hexokinase		Glucagon		Glucose 6-phosphate

Contd...

Contd...

Enzyme	Inducer	Repressor	Activator	Inhibitor
Glucokinase	Insulin	Glucagon		
Phosphofructokinase-1	Insulin	Glucagon^{Q}	5' AMP, fructose 6-phosphate, fructose 2,6-bisphosphate^{Q}, Pi	Citrate, ATP, glucagon
Pyruvate kinase	Insulin	Glucagon^{Q}	Fructose 1,6-bisphosphate, insulin	ATP, alanine, glucagon, norepinephrine
Pyruvate dehydrogenase	Insulin	Glucagon^{Q}	CoA, NAD^{+}, insulin^{Q}, ADP, pyruvate	Acetyl CoA^{Q}, NADH, ATP (fatty acids, ketone bodies)

Gluconeogenesis				
Enzyme	**Inducer**	**Repressor**	**Activator**	**Inhibitor**
Pyruvate carboxylase	Glucocorticoids, Glucagon, Epinephrine	Insulin	Acetyl CoA^{Q}	ADP
Phosphoenolpyruvate carboxykinase	Glucocorticoids, Glucagon, Epinephrine	Insulin		
Glucose 6-phosphatase	Glucocorticoids, Glucagon, Epinephrine	Insulin		

24. During prolonged fasting, rate of gluconeogenesis is determined by: (AIIMS May 2012)

a. Essential Fatty Acid in liver
b. Alanine in liver
c. Decreased c GMP
d. ADP in liver

Ans. b. Alanine in the liver

(Ref: Harpers 29/e p160; 30/e p186)

Major Substrates for Gluconeogenesis^{Q} (AI 97)

- Glucogenic Amino Acid (Alanine^{Q} is the major contributor)
- Lactate
- Glycerol

Propionate (Major contributor in Ruminants).

25. True about gluconeogenesis is/are: (PGI May 2013)

a. Prevent hypoglycemia during prolonged fasting
b. Occur in both muscle and liver
c. Fructose 2,6 bisphosphate stimulate it
d. Excess of acetyl CoA stimulate it
e. Carbon skeleton of amino acid is involved

Ans. a, d and e. *(Ref: Harper 29/e p187-191; 30/e p187)*

- Gluconeogenesis occur in liver and kidney not in the muscle
- Gluconeogenesis prevent hypoglycemia in prolonged fasting
- Fructose 2,6 Bisphosphate stimulate Glycolysis
- Excess Acetyl CoA is an allosteric activator of Pyruvate Carboxylase, a key enzyme of Gluconeogenesis
- Carbon skeleton of gluconeogenic amino acid are involved in gluconeogenesis.

26. Common enzyme for gluconeogenesis and glycolysis is: (Kerala 2007)

a. Glyceraldehyde 3 PO_4 dehydrogenase
b. Hexokinase
c. Pyruvate kinase
d. Pyruvate carboxylase

Ans. a. Glyceraldehyde 3 Phosphate dehydrogenase

- An enzyme that catalyze a reversible step in Glycolysis is common to both Glycolysis and Gluconeogenesis.

27. Phosphofructokinase-I is activated by all except: (NBE Pattern Q)

a. 5'AMP
b. Fructose 2, 6 Bisphosphate
c. Fructose 6 Phosphate
d. Citrate

Ans. d. Citrate. *(Ref: Harper 30/e p188 Table 19-1)*

Enzyme	Inducer	Repressor	Activator	Inhibitor
Hexokinase		Glucagon		Glucose 6-phosphate
Glucokinase	Insulin	Glucagon		
Phosphofructokinase-1	Insulin	Glucagon	5' AMP, fructose 6-phosphate, fructose 2,6-bisphosphate, Inorganic Phosphate	Citrate, ATP, glucagon
Pyruvate kinase	Insulin	Glucagon	Fructose 1, 6-bisphosphate, insulin	ATP, alanine, glucagon, norepinephrine

28. Not a substrate for gluconeogenesis: (NBE Pattern Q)

a. Acetyl CoA
b. Lactate
c. Glycerol
d. Propionyl CoA

Ans. a. Acetyl CoA *(Ref: Harper 29/e p185, 186)*

Substrates for Gluconeogenesis[Q]

- Glucogenic Amino Acid (Alanine[Q] is the major contributor)
- Lactate
- Glycerol
- Propionyl CoA.

29. Which of the following reactions takes place in two compartments? (NBE Pattern Q)

a. Gluconeogenesis
b. Glycolysis
c. Glycogenesis
d. Glycogenolysis

Ans. a. Gluconeogenesis *(Ref: Harper 30/e p188, 189)*

- Gluconeogenesis takes place in cytosol and mitochondria
- The Mitochondrial step is Pyruvate Carboxylase reaction.

Pathways taking place in two compartments are

- Heme Synthesis
- Urea Cycle
- Gluconeogenesis.

30. Glyconeogenic capability of cell is determined by the presence of: (PGI Dec 2005)

a. Pyruvate dehydrogenase
b. Glucose-6-phosphatase
c. Pyruvate carboxylase
d. Fructose 1,6- bisphosphatase
e. Pyruvate carboxykinase

Ans. b, c and d. *(Ref: Harper 30/e p188, 189)*

- Glyconeogenic capacity is determined by the presence of key enzymes of Gluconeogenesis.
- Gluconeogenesis is the process of synthesizing glucose or glycogen from noncarbohydrate precursors
- Pyruvate Dehydrogenase and Pyruvate carboxykinase are not enzymes of gluconeogenesis.

31. Step of Gluconeogenesis is: (NBE pattern Qn)

a. Pyruvate to Lactate
b. Glucose 6 Phosphate to Fructose 6 Phosphate

c. Pyruvate to Acetyl CoA
d. Oxaloacetate to Phosphoenolpyruvate

Ans. d. Oxaloacetate to PEP *(Ref: Harper 30/e p188, 189)*

- Pyruvate to lactate is a step in Anerobic Glycolysis
- Glucose 6 Phosphate to Fructose 6 Phosphate is a step in Glycolysis
- Pyruvate to Acetyl CoA is a step in aerobic oxidation of Glucose
- Oxaloacetate to PEP catalyzed by PEPCK is a step in gluconeogenesis.

32. Major contribution towards gluconeogenesis is by: AI 92

a. Lactate
b. Glycerol
c. Ketones
d. Alanine

Ans. d. Alanine *(Ref: Harper 30/e p188, 189)*

Alanine is the principal gluconeogenic amino acid

33. Glucose can be synthesized from all except: (AI 96)

a. Amino acids
b. Glycerol
c. Acetoacetate
d. Lactic acid

Ans. c. Acetoacetate *(Ref: Harper 30/e p188, 189)*

Substrates for Gluconeogenesis

- Glucogenic amino acids
- Lactate
- Glycerol

Acetyl CoA, Acetoacetate are not substrates for gluconeogenesis.

34. Gluconeogenesis does not occur significantly from ..... in humans: (AIIMS 92)

a. Lactate
b. Fatty acids
c. Pyruvate
d. Aminoacid

Ans. b. Fatty acids *(Ref: Harper 30/e p188, 189)*

Glycerol part of fat and Propionyl CoA from odd chain fatty acid oxidation are Gluconeogenic part of fat.

35. Acetyl CoA can be converted into all of the following except: (AI 2009)

a. Glucose
b. Fatty acids
c. Cholesterol
d. Ketone bodies

Ans. a. Glucose *(Ref: Harper 30/e p188,189)*

- Acetyl CoA is NEVER a substrate for gluconeogenesis
- Acetyl CoA is the starting material for Fatty acid and Cholesterol synthesis
- Acetyl CoA is an intermediate in Ketone body synthesis.

36. A genetic disorder renders fructose 1,6-bisphosphatase in liver less sensitive to regulation by fructose 2,6-biphosphate. All of the following metabolic changes are observed in this disorder except: AI 2004

a. Level of fructose 1,6-biphosphate is higher than normal
b. Level of fructose 1,6-biphosphate is lower than normal
c. Less pyruvate is formed
d. Less ATP is generated

Ans. b. Level of fructose 1, 6-biphosphate is lower than normal

- The action of Fructose 1,6 Bisphosphatase on Fructose 1,6 Bisphosphatase is decreasing its activity
- Here as the control of Fructose 1,6 BP on Fructose 1,6 Bisphosphatase is lost
- The enzyme is more active
- So the level of Fructose 1,6 Bisphosphate is lower than normal.

Glycogen Metabolism and Glycogen Storage Disorders

37. Why is Glucose 6 Phosphate in the cytoplasm of hepatocyte not acted upon by Glucose 6 Phosphatase as soon as it is formed? (AIIMS Nov 2015)

a. Thermodynamically possible only when gluconeogenesis occurs
b. Needs Protein Kinase for its activation
c. Enzyme is present in SER, Glucose 6-phosphate needs to be transported into SER
d. Steric inhibition of Phosphatase by albumin

Ans. c. Enzyme is present in SER, Glucose 6-phosphate need to be transported into SER *(Ref: Harper 30/e p178)*

- Glucose-6-phosphatase is in the lumen of the smooth endoplasmic reticulum
- Glucose-6-phosphate is transported to SER by a transporter called translocase to be acted by Glucose-6-phosphatase
- Genetic defects of the Glucose-6-phosphate transporter can cause a variant of type I glycogen storage disease.

Other options:

- Glycogenolysis provides blood glucose before Gluconeogenesis sets in in fasting state
- Glucose-6-phosphatase does not need Protein Kinase, but Glycogen Phosphorylase needs Protein Kinase for its activation.

38. The reasons for ketosis in von Gierke's disease are all except: (AIIMS Nov 2013)

a. Hypoglycemia
b. Oxaloacetate is necessary for gluconeogenesis
c. Low blood glucose less than 40 mg%
d. Fatty acid mobilization is low

Ans. d. Fatty acid mobilization is low

Glucose-6-phosphatase deficiency leading to **hypoglycemia.** Glucose-6-phosphate converted to Pyruvate. This is converted to Acetyl CoA. As **oxaloacetate is depleted because of using up of Oxaloacetate for Gluconeogenesis,** Acetyl CoA enter in to Ketone body synthesis. Hence Ketosis.

Glucose-6-phosphate also enters into HMP shunt pathway which leads to more production of Pentoses. Therefore more purine synthesis. Purines degrade to Uric Acid. Hence there is Hyperuricemia.

As there is hypoglycemia, ***fat is mobilized***. This also leads to more Acetyl CoA by Fatty acid oxidation. This increases Ketone body synthesis. Hence Ketosis.

39. A child with low blood glucose is unable to do glycogenolysis or gluconeogenesis. Which of the following enzymes is missing in the child? (AIIMS Nov 2012)

a. Fructokinase
b. Glucokinase
c. Glucose-6-phosphatase
d. Transketolase

Ans. c. Glucose-6-phosphatase

(Ref: Harper 29/e p179, 181 Table 19.2, 189; 30/e p177)

The answer should be an enzyme common to Glycogenolysis and Gluconeogenesis.

Glycogen Storage Disorders

Inborn errors of metabolism of Glycogen associated with accumulation or altered function of Glycogen in various organs are concerned with Glycogen metabolism.

Liver Glycogen Storage Disorder.

Type	Name	Enzyme efficiency	Characteristics
0	—	Glycogen synthase	Early morning drowsiness and fatigue, fasting hypoglycemia, and ketosis; early death [No Hepatomegaly]
Ia	von Gierke's disease	Glucose-6-phosphatase[α]	Glycogen accumulation in liver and renal tubule cells (Kidney Enlarged) hypoglycemia; elevated blood lactate, cholesterol, triglyceride, and uric acid levels
Ib	—	Endoplasmic reticulum glucose-6-phosphate transporter/ translocase	Same as type Ia, with additional findings of neutropenia and impaired neutrophil function Recurrent Bacterial Infection, Inflammatory Bowel Disease
III	Limit dextrinosis, Forbe's or Cori's disease	Liver and muscle debranching enzyme(Amylo 1,6 Glucosidase)	Fasting hypoglycemia hepatomegaly in infancy accumulation of characteristic branched polysaccharide (limit dextrin), muscle weakness, elevated transaminase levels; liver symptoms can progress to liver failure later in life
IV	Amylopectinosis, Andersen's disease	Branching enzyme	Hepatosplenomegaly Accumulation of polysaccharide with few branch points Failure to thrive, hypotonia, hepatomegaly, splenomegaly, progressive cirrhosis (death usually before 5th yr), elevated transaminase levels
VI	Hers' disease	Liver phosphorylase	Hepatomegaly
VIII		Liver phosphorylase kinase	
	Fanconi Bickel Syndrome QNBE pattern	Glucose transporter 2 (GLUT-2)	Failure to thrive, rickets, hepatorenomegaly, proximal renal tubular dysfunction, impaired glucose and galactose utilization

40. In which of the following tissues is glycogen incapable of contributing directly to blood glucose: (AI-2008)

a. Liver
b. Muscle

c. Both
d. None

Ans. b. Muscle *(Ref: Harper 29/e p180; 30/e p177, 178)*

Differences between Liver Glycogen and Muscle Glycogen.

Features	Liver	Muscle
Total Body Glycogen content	Less	Highest
Percentage by tissue weight	Highest	Less
Regulation of blood glucose	Contributes to blood glucose	Does not contribute to blood glucose

41. In humans carbohydrates are stored as: (Kerala 2006)

a. Glucose
b. Glycogen
c. Starch
d. Cellulose

Ans. b. Glycogen *(Ref: Harper 30e p154)*

- Glycogen is the storage polysaccharide in animals and is sometimes called animal starch.

42. Glycogen is released from the muscle due to increased cAMP due to:

a. Epinephrine
b. Thyroxine
c. Glucagon
d. Growth hormone

Ans. a. Epinephrine *(Ref: Harper 30/e p180, 181)*

Differences between Muscle and Liver in the regulation of Glycogen Metabolism

- Epinephrine acts in Muscle and Liver whereas Glucagon acts only in the Liver
- In the muscle there is **cAMP-independent activation of glycogenolysis**[Q]
- By the stimulation of a Ca^{2+}/calmodulin-sensitive phosphorylase kinase
- Phosphorylate Glycogen Phosphorylase in the muscle
- Favor glycogenolysis
- Muscle phosphorylase can be activated without phosphorylation
- Muscle Phosphorylase has a binding site for 5′AMP
- 5′AMP is an allosteric activator without phosphorylation
- Favor Glycogenolysis.

43. Pancreatic alpha amylase:

a. Convert starch to glycogen
b. Hydrolyses starch to limit dextrins
c. Hydrolyses Starch to Monosaccharides
d. Convert maltose to glucose

Ans. c. Hydrolyses Starch to limit dextrins. *(Ref: Harper 30/e p538)*

44. A 5 years old boy presents with hepatomegaly, hypoglycaemia, ketosis. The diagnosis is

a. Mucopolysaccharidosis
b. Glycogen storage disorder
c. Lipopolysaccharidosis
d. Diabetes mellitus

Ans. b. Glycogen storage disorder

(Ref: Nelson 20/e Chapter 715 Defects in metabolism of Carbihydrates)

- Patients with type I GSD may present in the neonatal period with hypoglycemia and lactic acidosis
- These children often have doll-like faces with fat cheeks, relatively thin extremities, short stature, and a protuberant abdomen that is due to massive hepatomegaly; the kidneys are also enlarged, whereas the spleen and heart are normal
- The biochemical hallmarks of the Type I a GSD (von Gierke's) disease are hypoglycemia, lactic acidosis, hyperuricemia, and hyperlipidemia.

45. Glycogen phosphorylase can be regulated by all the following except: (AIIMS Nov 2015)

a. cAMP
b. Calmodulin
c. Protein Kinase A
d. Glycogenin

Ans. d. Glycogenin *(Ref: Harper 30/e p180-182)*

Regulation of Glycogen metabolism at Glycogen Phosphorylase

- cAMP activates Glycogen Phosphorylase by cAMP-dependent Protein Kinase
- cAMP-independent Calcium/Calmodulin-sensitive Phosphorylase. Kinase also activates Glycogen Phosphorylase.

Glycogenin is a protein on which initial glucose is added in the synthesis of Glycogen.

46. Cofactor for Glycogen Phosphorylase: (AIIMS Nov 2015)

a. Thiamine Pyrophosphate
b. Pyridoxal Phosphate
c. Citrate
d. FAD

Ans. b. Pyridoxal Phosphate. *(Ref: Harper 30/e p178)*

47. Pompe's disease is due to deficiency of: **(NBE Pattern Q)**

a. Debranching enzyme
b. Muscle phosphorylase
c. Acid maltase
d. Branching enzyme

Ans. c. Acid maltase. *(Ref: Harper 30/e p178)*

LIVER GLYCOGEN STORAGE DISORDERS		
Type	**Name**	**Enzyme efficiency**
0	—	Glycogen synthase
Ia	von Gierke's disease	Glucose-6-phosphatase[Q]
Ib	—	Endoplasmic reticulum glucose-6-phosphate transporter
III	Limit dextrinosis, Forbe's or Cori's disease	Liver and muscle debranching enzyme (Amylo 1,6 Glucosidase)
IV	Amylopectinosis, Andersen's disease	Branching enzyme
VI	Hers' disease	Liver phosphorylase
VIII		Liver phosphorylase kinase
	Fanconi Bickel Syndrome[QNBE pattern]	Glucose transporter 2 (GLUT-2)
MUSCLE GLYCOGEN STORAGE DISORDER		
II	Pompes's Disease (Belongs to lysosomal storage disorder)	Lysosomal α1,4 and α1,6 glucosidase (acid maltase)[Q]
	Danon disease	Lysosome-associated membrane protein 2 (LAMP2)
V	McArdle's syndrome	Muscle phosphorylase[Q]
VII	Tarui's disease	Muscle and erythrocyte phosphofructokinase 1

48. Glycogen storage disorder(s) is/are:
a. Niemann-Pick Disease **(PGI Nov 2014)**
b. Gaucher Disease
c. Tay-Sacks disease
d. Pompe's disease
e. McArdle's disease

Ans. d. Pompe's disease, **e.** McArdle's disease.
(Ref: Nelson 20/e Chapter Defects in the Metabolism of Carbohydrates)

49. How many hours for depletion of glycogen: **(NBE pattern Qn)**

a. 9
b. 18
c. 24
d. 48

Ans. b. 18 hours *(Ref: Harper 30/e p149)*

- Liver and Muscle Glycogen exhausted by 18 hours of fasting.

50. In the fed state, major fate of glucose-6-phosphate in tissues is: **(AIIMS may 93)**

a. Storage as fructose
b. Storage as glyceraldehyde-3-phosphate
c. Enters HMP shunt via ribulose-5-phosphate
d. Storage as glycogen

Ans. d. Storage as glycogen *(Ref: Harper 30/e p148)*

- The uptake of glucose into the liver GLUT 2 is independent of insulin
- In the well fed state, the concentration of glucose entering the liver increases, so does the rate of synthesis of glucose-6-phosphate
- This is in excess of the liver's requirement for energy-yielding metabolism. So it is used mainly for synthesis of glycogen
- In both liver and skeletal muscle, insulin acts to stimulate glycogen synthetase and inhibit glycogen phosphorylase.

51. Which of the following is a debranching enzyme: **(AIIMS 90)**

a. Glycogen synthetase
b. Glucose-6-phosphatase
c. Amylo 1,6 glucosidase
d. Amylo 1,4-1,6 transglycosylase

Ans. c. Amylo 1,6 Glucosidase. *(Ref: Harper 30/e p178)*

52. Sequence of events in glycogenolysis: **(PGI June 97, Dec 96)**

a. Phosphorylase, glucan transferase, debranching, phosphorylase
b. Debranching, phosphorylase, transferase, phosphorylase
c. Transferase, phosphorylase, debranching, phosphorylase
d. Any of the above

Ans. a. Phosphorylase, glucan transferase, debranching, phosphorylase *(Ref: Harper 30/e page 180,181)*

Steps of Glycogenolysis

Breaking of α 1,4 linkage

- Glycogen phosphorylase cleave the **α 1,4 linkage**
- Releases Glucose 1 Phosphate, NOT free Glucose
- Glycogen Phosphorylase stops its action when it is at least 4 glucose residues from a branch point.

Removal of Branches

By a bifunctional enzyme:

- First part is a α-1, 4 α 1,4 Glucan transferase
  - Transfer trisaccharide residue to another forming a new α 1, 4 linkage
- Second part is α1, 6 Glucosidase (Amylo 1,6 Glucosidase). Hydrolyse the branching point
  - Releases free Glucose, NOT Glucose 1 Phosphate.

Conversion of Glucose 1 Phosphate to free Glucose

- Glucose 1 Phosphate to Glucose-6-phosphate by Phosphoglucomutase
- Glucose-6-Phosphate to Glucose by Glucose-6-phosphatase
- Glucose-6-phosphatase is present in the smooth endoplasmic reticulum
- A transporter is required for the transport of Glucose-6-phosphate from SER to cytoplasm
- Defect in the Glucose-6-phosphate transporter lead to Type Ib Glycogen Storage disorder.

53. Muscles are not involved in which glycogen storage disease: (PGI Dec 97)

a. I
b. II
c. III
d. IV

Ans a. I

(Ref: Nelson 20/e Defects in the metabolism of Carbohydrates)

Glycogen Storage Disorder II (Limit dextrinoses) and GSD IV (Anderson disease) are liver GSD with muscle involvement.

Muscle Glycogen Storage Disorders

Type	Name	Enzyme defect
II	Pompe's Disease (Belongs to lysosomal storage disorder)	Lysosomal α1,4 and α1,6 glucosidase (acid maltase)[α]
	Danon disease	Lysosome-associated membrane protein 2 (LAMP2)
V	McArdle's syndrome	Muscle phosphorylase[α]
VII	Tarui's disease	Muscle and erythrocyte phosphofructokinase 1

54. An infant has hepatosplenomegaly, hypoglycemia, hyperlipidemia, acidosis and normal structured glycogen deposition in liver. What is the diagnosis: (PGI June 01)

a. Her's disease
b. von Gierke's disease
c. Cori's disease
d. Anderson's disease
e. Pompe's disease

Ans. b. von Gierke's Disease

(Ref: Nelson 20/e Defects in the metabolism of Carbohydrates)

Clinical Picture of von Gierke's Disease

Clinical presentation

- **Most commonly present at 3–4 months of age with**
- Doll-like facies with fat cheeks
- Relatively thin extremities
- Short stature, Protuberant abdomen
- Massive Hepatomegaly
- **Kidneys are also enlarged**
- **No Splenomegaly**
- **Plasma may be milky due to associated hypertriglyceridemia.**

Type Ib has additional features of recurrent bacterial infection due to neutropenia and impaired neutrophil dysfunction.

The biochemical hallmarks are:

- Hypoglycemia
- Lactic Acidosis
- Hyperlipidemia
- Hyperuricemia.

Long-term effects of Type I GSD are PCOD, Pancreatitis, Hepatic Adenomas, Pulmonary hypertension, Osteopenia, Renal disease.

55. Glycogen storage diseases include all the following except: (PGI Dec 01)

a. von Gierke's disease
b. Fabry's disease
c. McArdle's disease
d. Fragile X syndrome
e. Krabbe's disease

Ans. b, d, e.

(Ref: Nelson 20/e Defects in the metabolism of Carbohydrates)

- **Fabry's Disease and Krabbe's Disease are Sphingolipidoses.**

56. The cause of hyperuricemia and gout in Glucose-6-phosphatase deficiency is: (AIIMS Nov 01)

a. More formation of pentose
b. Decreased availability of glucose to tissues
c. Increased accumulation of sorbitol
d. Impaired degradation of free radicals

Ans. a. More formation of pentoses

(Ref: Nelson 20/e Defects in the metabolism of Carbohydrates)

Biochemical defect in von Gierke's

Glucose 6 Phosphatase deficiency → Glucose 6 Phosphate accumulate → channeled to Pentose Phosphate pathway → more Pentoses → To Purine Synthesis → So Uric acid the degradation product Purine accumulate.

HMP Pathway

57. Metabolites in HMP Shunt are all except: (AI 95)

a. Glycerol-3-phosphate
b. Sedoheptulose-7-phosphate
c. Glyceraldehyde-3-phosphate
d. Xylulose-5-phosphate

Ans. a. Glycerol 3 Phosphate *(Ref: Harper 30/e p197)*

Metabolites in HMP Shunt Pathway

- Glucose-6-phosphate
- 6-Phosphogluconate
- Ribulose-5-Phosphate
- Xylulose-5-Phosphate
- Ribose-5-Phosphate
- Glyceraldehyde-3-Phosphate
- Sedoheptulose-7-Phosphate
- Fructose-6-phosphate
- Erythrose 4 Phosphate.

58. NADPH is produced by: (AIIMS Nov 2003)

a. Glycolysis
b. Citric acid cycle
c. HMP shunt
d. Glycogenesis

Ans. c. HMP shunt *(Ref: Harper 30/e p197)*

Key points to remember in HMP (Pentose Phosphate) Pathway

- Main source of NADPH[Q2012] and Pentoses
- Two transketolation and one transaldolation reactions are involved
- No ATP is produced[Q]
- CO_2 is produced in this pathway and not in glycolytic pathway[Q] DNB 01)
- Deficiency of Glucose-6-phosphate dehydrogenase is a major cause of acute hemolysis in erythrocytes
- NADPH is used for **reductive biosynthetic pathways**, like fatty acid synthesis, steroid synthesis, Amino acids by Glutamate dehydrogenase
- NADPH is required for regeneration of reduced Glutathione, that clears free radicals from erythrocytes and lens.

59. Reduced NADPH is produced from which pathway: (NBE Pattern Q)

a. Krebs cycle
b. Anerobic glycolysis
c. Uronic acid pathway
d. Hexose monophosphate pathway

Ans. d. HMP pathway. *(Ref: Harper 30/e p197)*

60. Which of the following metabolic pathways does not generate ATP: (AI 2008)

a. Glycolysis
b. TCA Cycle
c. Fatty Acid Oxidation
d. HMP Pathway

Ans. d. HMP Pathway. *(Ref: Harper 29/e p199; 30/e p200)*

Metabolic pathway	Net ATP yield
Anerobic Glycolysis	2
Aerobic Glycolysis	7
Aerobic oxidation of 1 mol of glucose	32
Palmitic Acid Oxidation	106
Citric Acid Cycle	10
Rapaport-Luebering Cycle	0
HMP Pathway	0

61. Severe thiamine deficiency is associated with: (JIPMER 2013)

a. Decreased RBC transketolase activity
b. Increased clotting time
c. Decreased RBC transaminase activity
d. Increased xanthic acid excretion

Ans. a. Decreased RBC transketolase

(Ref: Harper 30/e p203, 204)

Thiamine is a coenzyme of Erythrocyte transketolase so its activity is decreased if Thiamine deficiency.

Other Metabolic Pathways of Glucose

62. Products of uronic acid pathway in human beings are all except: (NBE pattern Qn)

a. Vitamin C
b. Glucuronic acid
c. Pentoses
d. NADH

Ans. a. Vitamin C *(Ref: Harper 30/e p202, 203)*

- Uronic acid pathway cannot synthesize Vitamin C in humans and higher primates because of lack of L-Gulanolactone Oxidase.

63. Uronic acid pathway is not involved in: **(NBE Pattern Qn)**

a. Conjugation of bilirubin
b. GAG Synthesis
c. Vitamin C Synthesis
d. Biotransformation

Ans. c. Vitamin C Synthesis *(Ref: Harper 30/e p202, 203)*

- Glucuronic acid is involved in the conjugation of Bilirubin
- Uronic acid and Amino Sugar are the repeating disaccharide unit in the Glycosaminoglycans
- Glucuronidation is a Phase II Xenobiotic reactions (Biotransformation)
- Uronic acid pathway cannot synthesize Vitamin C in humans and higher primates because of lack of L-Gulonolactone Oxidase.

Fructose Metabolism and Disoders

64. Hereditary Fructose Intolerance is due to deficiency of: **(JIPMER 2014)**

a. Aldolase B
b. Aldolase A
c. Fructokinase
d. Sucrase

Ans. a. Aldolase B *(Ref: Nelson 20/e Chapter Defects in metabolism of carbohydrates)*

- **Hereditary Fructose Intolerance due to deficiency of Aldolase B**
- **Essential Fructosuria due to deficiency of Fructokinase.**

65. False about hereditary fructose intolerance: **(AIIMS June 98)**

a. Deficiency of fructose 1-phosphate aldolase
b. Accumulation of fructose 1-phosphatein tissues
c. Hyperglycemia
d. Liver and kidneys are involved

Ans. c. Hyperglycemia.

(Ref: Nelson 20/e Chapter Defects in metabolism of carbohydrates)

Biochemical defect of Hereditary Fructose Intolerance

- Deficiency of Fructose-1,6-Bisphosphate Aldolase (Aldolase B)
- The gene for aldolase B is on chromosome 9q22.3
- Deficiency of this enzyme activity causes a rapid accumulation of fructose-1-phosphate and initiates severe toxic symptoms when exposed to fructose.

Clinical manifestations

- Patients with HFI are asymptomatic until fructose or sucrose (table sugar) is ingested (usually from fruit, fruit juice, or sweetened cereal)
- Symptoms may occur early in life, soon after birth if foods or formulas containing these sugars are introduced into the diet
- Early clinical manifestations resemble galactosemia and include jaundice, hepatomegaly, vomiting, lethargy, irritability, and convulsions, hypoglycemia
- Acute fructose ingestion produces symptomatic hypoglycemia. If the intake of fructose persists, hypoglycemic episodes recur, and liver and kidney failures progress, eventually leading to death
- Chronic ingestion results in failure to thrive.

Galactose Metabolism and Disoders

66. Galactosemia enzyme defect:

a. Fructokinase
b. Glucokinase
c. Galactose-1-Phosphate Uridyl Transferase
d. Glucose-6-Phosphatase

Ans. c. Galactose 1 Phosphate Uridyl Transferase

(Ref: Harper 29/e p206)

Galactosemia

Enzyme Deficiency (AIIMS Nov 2011, May 2013)

- **Galactose-1-Phosphate Uridyl Transferase [Classic Galactosemia]**
- **Galactokinase**
- UDP Hexose 4 Epimerase

The newborn infant receives high amounts of lactose, which consists of equal parts of glucose and galactose. Without the Galactose-1-Phosphate Uridyl Trasferase enzyme, the infant is unable to metabolize galactose-1-phosphate, the accumulation of which results in injury to kidney, liver, and brain.

Clinical features

- Classic galactosemia is a serious disease with onset of symptoms **typically by the 2nd half of the First week of life**
- With jaundice, vomiting, seizures, lethargy, irritability, feeding difficulties, poor weight gain or failure to regain birthweight
- Hepatomegaly oil drop cataracts due to accumulation of Galactictol/dulcitol
- Hepatic failure
- Mental retardation
- Patients with galactosemia are at increased risk for Escherichia coli neonatal sepsis.

67. A newborn baby refuses breast milk since the second day of birth, vomits on force-feeding but accepts glucose-water, develops diarrhea on third day, by fifth day, she is jaundiced with liver enlargement and eyes show cataract. Urinary-reducing sugar was positive but blood glucose estimated by glucose oxidation method was found low. The most likely cause is deficiency of: (AIIMS May 03)

a. Galactose-1-phosphate uridyl transferase
b. Beta galactosidase
c. Glucose-6-phosphate
d. Galactokinase

Ans. a. Galactose-1-phosphate uridyl transferase

(Ref: Nelson 20/e Chapter Defects in metabolism of carbohydrates)

- Classic galactosemia is a serious disease with onset of symptoms typically by the 2nd half of the 1st wk of life
- With jaundice, vomiting, seizures, lethargy, irritability, feeding difficulties, poor weight gain or failure to regain birthweight
- Hepatomegaly Oil drop cataracts, Hepatic failure
- Mental retardation
- Patients with galactosemia are at increased risk for *Escherichia coli* neonatal sepsis.

Other options:

- **Beta Galactosidase is lactase**
- **In Galactokinase deficiency, the sole manifestation is Cataract**
- **Glucose-6-phosphate deficiency is von Gierke's disease.**

68. A child presents with hepatomegaly and bilateral lenticular opacities. Deficiency of which of the following enzymes will not cause such features:

a. Galactose-1-phosphate uridyl transferase
b. UDP galactose 4-epimerase
c. Galactokinase
d. Lactase

Ans. d. Lactase

(Ref: Nelson 20/e Chapter Defects in metabolism of carbohydrates)

69. True regarding galactosemia: (PGI Dec 01)

a. Mental retardation occurs
b. Absent disaccharidase in intestine
c. Defect in epimerase
d. Defect in galactose 1-phosphate uridyl transferase

Ans. a, c, d.

(Ref: Nelson 20/e Chapter Defects in metabolism of carbohydrates)

Integration of Metabolism (Fed State and Fasting State)

70. Which is used for energy: (PGI May 2013)

a. Ketone bodies
b. Glucose
c. Free fatty acids
d. Creatinine
e. Collagen

Ans. a, b, c.

(Ref: Harper 29/e p161 Table 16.3; 30/e p150 Table 14.3)

Metabolic Fuels for Different Organs

Organ	Major metabolic fuels
Liver	Free fatty acids, glucose (in fed state), lactate, glycerol, fructose, amino acids, alcohol
Brain	Glucose, amino acids, ketone bodies in prolonged starvation
Heart[Q]	Ketone bodies, free fatty acids, lactate, chylomicron and VLDL triacylglycerol, some glucose
Adipose tissue	Glucose, chylomicron and VLDL triacylglycerol
Fast Twitch Muscle	Glucose, glycogen
Slow Twitch Muscle	Ketone bodies, chylomicron and VLDL triacylglycerol
Kidney	Free fatty acids, lactate, glycerol, glucose
Erythrocyte	Glucose

71. All the following are increased in starvation except: (PGI Nov 2012)

a. Lipolysis
b. Ketogenesis
c. Gluconeogenesis
d. Glycogenesis
e. Glycogenolysis

Ans. d. Glycogenesis

Glycogenesis is increased in well fed state.

72. Which enzyme is active when insulin glucagon ratio is low? (AIIMS Nov 2013)

a. Glucokinase
b. Hexokinase
c. Glucose-6-phosphatase
d. Pyruvate Carboxylase

Ans. c. Glucose-6-phosphatase, **d.** Pyruvate Carboxylase

(Ref: Harper 30/e p188 Table 19.1)

Insulin: Glucagon ratio is more when body is in the fasting state. Key Enzymes of Gluconeogenesis will be active. So, Pyruvate Carboxylase and Glucose 6 Phosphatase are active.

73. Substrate used by RBC in fasting state is: (May AIIMS 2015)

a. Glucose
b. Amino Acids
c. Ketone body
d. Fatty acid

Ans. a. Glucose *(Ref: Harper 30/e p150 Table 14.3)*

Organ	Major metabolic fuels
Liver	Free fatty acids, glucose (in fed state), lactate, glycerol, fructose, amino acids, alcohol
Brain	Glucose, amino acids, **ketone bodies in prolonged starvation**
Heart[α]	Ketone bodies, free fatty acids, lactate, chylomicron and VLDL triacylglycerol, some glucose
Adipose tissue	Glucose, chylomicron and VLDL triacylglycerol
Fast Twitch Muscle	Glucose, glycogen
Slow Twitch Muscle	Ketone bodies, chylomicron and VLDL triacylglycerol
Kidney	Free fatty acids, lactate, glycerol, glucose
Erythrocyte	Glucose

74. Lactic acidosis in thiamine deficiency is due to which enzyme dysfunction? (AIIMS May 2015)

a. Phosphoenol Pyruvate Carboxykinase
b. Pyruvate Dehydrogenase
c. Pyruvate Carboxylase
d. Aldolase

Ans. b. Pyruvate Dehydrogenase *(Ref: Harper 30/e p174)*

In Thiamine deficiency, PDH reaction is defective. So Pyruvate is converted to lactic acid.

Causes of inhibition of PDH leading to lactic acidosis:

- Inherited PDH deficiency
- Thiamine deficiency
- Alcoholics due to thiamine deficiency
- Arsenite and Mercury poisoning.

3

Section | Lipids

CHAPTERS

6. Chemistry of Lipids and Biomembranes
7. Metabolism of Lipids

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6 Chemistry of Lipids and Biomembranes

Topics Included

- Classification of Lipids
- Fatty Acids
- Triacylglycerol
- Phospholipids
- Glycolipids
- Biomembranes

DEFINITION

Lipids are heterogenous group of compounds relatively insoluble in water and freely soluble in nonpolar organic solvents—ether, chloroform.

Unlike Carbohydrates and Proteins they are not chemically related, but they are physically related.

- *Carbohydrates:* Polymer of Monosaccharide
- *Proteins:* Polymer of Amino Acids.

Lipids are not true polymers, but mixture of chemically unrelated substances.

CLASSIFICATION OF LIPIDS

Bloor's Classification

Simple Lipids

They are esters of fatty acid with alcohol

Fatty Acid + Alcohol = Esters of fatty acid

They are divided into

- Fats and oils
- Waxes.

Fats and oils

They are esters of fatty acid with alcohol, glycerol.

Fatty Acid + Glycerol

Fats and oils are the same except that fats are solid at room temperature, and oils are liquid at room temperature.

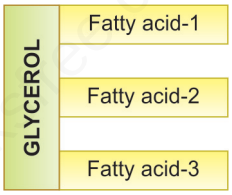


Fig. 6.1: Diagrammatic representation of triacylglycerol

Waxes

They are esters of fatty acid with higher molecular weight monohydric alcohol other than glycerol, e.g. Bee wax, Lanolin.

Fatty Acid + High molecular weight alcohol other than glycerol

Complex Lipids

They are esters of fatty acid with alcohols having additional groups like Phosphoric acid, Carbohydrates, Proteins etc.

Fatty Acid + Alcohol (Glycerol/Sphingosine) + additional groups

Depending on the additional groups present they are again classified into

- Phospholipids
- Glycolipids
- Other complex lipids like Sulfolipids, Lipoproteins, Amino lipids.

Phospholipids

Lipids containing phosphoric acid residue and nitrogenous base in addition to glycerol and fatty acid.

Fatty Acid + Alcohol (Glycerol/Sphingosine) + Phosphate + Nitrogenous base

Phospholipids divided into

- *Glycerophospholipids:* The alcohol in this group is Glycerol, e.g. Lecithin, Cephalin
- *Sphingophospholipids:* The alcohol in this group is an amino alcohol Sphingosine, e.g. Sphingomyelin.

Glycolipids or Glycosphingolipids[Q]

- Lipids containing carbohydrate apart from Fatty acid and Alcohol (usually Sphingosine)
- **Fatty Acid + Alcohol (Sphingosine) + Carbohydrate,** e.g. Cerebroside, Ganglioside.

Lipoproteins

- Lipids complexed with proteins, e.g. Low density Lipoprotein (LDL), High Density Lipoprotein (HDL).

Derived Lipids or Precursor Lipids

Compounds which are derived from the above group of Lipids, e.g. Fatty acids, Glycerol, Cholesterol.

Miscellaneous Lipids

Vast number of lipids which are not classified under any of the above groups, e.g. Squalene, Carotenoids, Vitamin E, Vitamin K.

FATTY ACIDS

They are aliphatic carboxylic acid.

General Structural Formula of Fatty acid—R-COOH

- R is the Aliphatic Hydrocarbon chain.
- R group accounts for the nonpolar nature of fat.

Numbering the Carbon Atoms in Fatty Acids

- Carbon atoms are numbered from the carboxyl carbon (carbon no. 1)
- The carbon atoms adjacent to the carboxyl carbon (nos. 2, 3, and 4) are also known as the α, β, and γ carbons, respectively, and the terminal methyl carbon is known as the ω-or n-carbon.

Conventions Used to Indicate the Positions of Double Bonds

- Various conventions use for indicating the number and position of the double bonds
- Δ^9 indicates a double bond between carbons 9 and 10 of the fatty acid counting from the carboxyl end
- ω9 indicates a double bond on the ninth carbon counting from the ω-carbon.

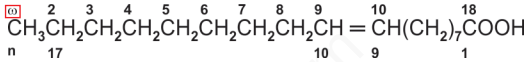


Fig. 6.2: Nomenclature of number and position of double bond of unsaturated fatty acid

Classification of Fatty Acid

Depending on the Chain Length

- Short Chain Fatty Acid (C2–C6)
- Medium Chain Fatty Acid (C8–C14)
- Long Chain Fatty Acid (≥ C16).

Depending on the Presence of Double Bond

- *Saturated fatty acid:* No double bond in the Hydrocarbon chain
- *Unsaturated fatty acid:* Double bonds are present in the Hydrocarbon chain.

Depending on the number of double bonds present unsaturated fatty acid is again classified into:

- *Monounsaturated Fatty Acid:* Only one double bond is present
- *Polyunsaturated Fatty Acid:* More than one double bonds are present.

Common saturated fatty acids and their sources

Saturated fatty acid	Source
Acetic Acid (2C)	Vinegar
Butyric Acid (4C)	Butter
Valeric Acid (5C)	Butter
Caproic Acid (6C)	Butter and Coconut milk
Lauric Acid (12C)	Coconut milk
Myristic Acid (14C)	Coconut milk
Palmitic Acid (16C)	Body Fat
Stearic Acid (18C)	Body Fat

Common Unsaturated Fatty Acids

Number of C atoms and number and position of common double bonds	Family	Common name	Occurrence[Q]
Monoenoic acids (one double bond)			
16:1; 9	ω7	Palmitoleic	In nearly all fats
18:1; 9	ω9	Oleic	High in olive oil

Contd...

Contd...

Number of C atoms and number and position of common double bonds	Family	Common name	Occurrence[Q]
18:1; 9	ω9	Elaidic	Hydrogenated and ruminant fats
Dienoic acids (two double bonds)			
18:2; 9, 12	ω6	Linoleic	Corn, peanut, cottonseed, soybean
Trienoic acids (three double bonds)			
18:3; 6, 9, 12	ω6[Q]	γ-Linolenic (GLA)	Oil of evening primrose, borage oil; linseed oil
18:3; 9, 12, 15	ω3[Q]	α-Linolenic	Linseed oil
Tetraenoic Acid (four double bonds[Q])			
20:4; 5, 8, 11, 14	ω6	Arachidonic	Found in animal fats; important component of phospholipids in animals
Pentaenoic acids (five double bonds)			
20:5; 5, 8, 11, 14, 17	ω3	Timnodonic (Eicosapentaenoic)	Important component of fish oils, e.g. cod liver, mackerel, menhaden, salmon oils
Hexaenoic acids (six double bonds)			
22:6; 4, 7, 10, 13, 16, 19	ω3	Cervonic (Docosahexaenoic) (DHA)	Fish oils, phospholipids in brain, Breast milk[Q]

NB: This table is very important, Numerous questions can be asked.

Significance of Medium Chain Fatty Acid
- Absorbed directly into the Blood
- Do not need Carnitine for transport into Mitochondria
- No effect on Atherosclerosis.

ESSENTIAL FATTY ACID

The fatty acids that are required by humans, but are not synthesized in the body hence need to be supplied in the diet are known as essential fatty acid (EFA). Humans lack the enzymes that can introduce double bond beyond 9th Carbon.

They are Polyunsaturated Fatty Acid namely:
- Linoleic Acid
- Alpha Linolenic Acid
- Arachidonic Acid is considered as semi essential fatty Acid as it can be synthesized from Linoleic Acid.

Functions of Essential Fatty Acid
- They are integral components of membrane structure, often in the 2 position of phospholipids
- Eicasanoids are synthesized from Arachidonic Acid. Essential fatty acids are needed for the synthesis of Arachidonic Acid
- Lower the risk of Cardiovascular Diseases
- Lower the risk of Fatty liver.

Deficiency of Essential Fatty Acid
- Skin: Acanthosis and Hyperkeratosis
- Fatty liver
- Swelling of mitochondrial membrane and reduction in efficiency of oxidative phosphorylation
- Decrease in fibrinolytic activities.

OMEGA CLASSIFICATION OF FATTY ACID

In this type of classification position of double bond is counted from the methyl end (ω carbon atom). Depending on the position of first double bond from the terminal end, the fatty acids are again classified into.

ω3 Series	• α Linolenic Acid • Timnodonic Acid (Eicosa pentaenoic Acid) • Cervonic Acid (Docosa Hexaenoic Acid) (DHA)
ω6 Series	• Linoleic Acid • Linolenic Acid (GLA) • Arachidonic Acid
ω9 Series	• Oleic Acid • Elaidic Acid

Significance of ω3 Fatty Acid
- Decrease the risk of Cardiovascular Disease
- Appear to replace arachidonic acid in platelet membranes
- Lower the production of Thromboxane and tendency of the platelet aggregation
- Decrease Serum Triglycerides
- Important for Infant Development
- Lower the risk of various mental illness (Depression, ADHD)
- Lower the risk of chronic degenerative diseases such as Cancer, Rheumatoid Arthritis, and Alzheimer's Disease.

DOCOSA HEXAENOIC ACID (DHA)

- *Sources:* Human milk, Fish liver oils, Algal oils
- Synthesized in the body from α Linolenic acid.
- Highest concentration of DHA found in retina, cerebral cortex, sperms.
- *Functions:* Needed for the development of fetal brain and retina
- DHA is supplied transplacentally and through breast milk.
- *Clinical significance:* Low DHA is associated with increased risk of Retinitis Pigmentosa.

ISOMERISM IN FATTY ACIDS

Cis-trans Isomerism

When similar groups occur on same side of the double bond it is called cis isomer and when similar group occur on opposite side it is called trans isomer.

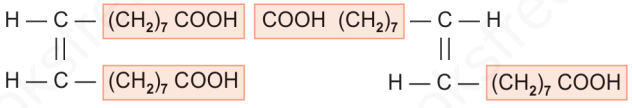
Biological Significance of Cis isomers

All naturally occurring amino acids are cis isomers.

Cis isomers increases the fluidity of biological membranes.

Trans fatty Acids

- Present in dairy products and partially hydrogenated edible oils (e.g. Margarine)
- They are used in food industry to improve the shelf life
- Trans fatty acids are present in high amounts in processed foods, fast foods and bakery items and fried foods
- Trans fatty acid compete with essential fatty acid, hence exacerbate essential fatty acid deficiency
- Consumption of trans fatty acid for long terms may raise the risk factor for cardiovascular diseases like Atherosclerosis and Coronary Artery disease and Diabetes mellitus.



Oleic acid-cis isomer Elaidic acid trans isomer

Fig. 6.3: Cis and trans isomer of elaidic acid

DIETARY SOURCES OF FATTY ACID

Fats/Oil	SFA (%)	MUFA (%)	Linoleic acid (%)	Alpha Linolenic acid (%)
High Medium chain and Short chain fatty acid				
Coconut	92	6	2	—
Palm kernel	83	15	2	—

Contd...

Contd...

Fats/Oil	SFA (%)	MUFA (%)	Linoleic acid (%)	Alpha Linolenic acid (%)
Butter/Ghee	68	29	2	1
High SFA and MUFA				
Palmolein	39	46	11	< 0.5
High MUFA and Moderate Linoleic acid				
Ground nut	19	41	32	< 0.5
Rice bran	17	43	38	1
Sesame	16	41	42	< 0.5
High linoleic acid				
Cotton seed	24	29	48	1
Corn	12	35	50	1
Safflower	9	13	75	—
Sunflower	12	22	62	—
High Linoleic acid and alpha linolenic acid				
Soybean	14	24	53	7
Canola	6	60	22	10
Mustard/Rapeseed	4	65	15	14
Flax-Seed	10	21	16	5
High trans fatty acid				
Vanaspati	46	49	4	—

Dietary Sources of Fatty Acids

- Highest content of MUFA in Mustard/Rapeseed oil
- Highest content of Medium chain fatty acid in Coconut oil
- Highest content Linoleic acid in Safflower oil
- Highest content alpha linolenic acid in Flax seed oil
- **Highest amount of PUFA is present in Safflower oil**
- **Second highest source of PUFA is Sunflower oil**
- **Least source of PUFA is Coconut oil**
- **Richest source of medium chain fatty acid is Coconut oil**
- **Fatty acid present in human milk is Docosa Hexaenoic Acid (Cervonic Acid).**
- **Fatty acid present in the fish oils are**
  - Timnodonic Acid
  - Clupanodonic Acid
  - Cervonic Acid.

TRIACYLGLYCEROL (TAG)

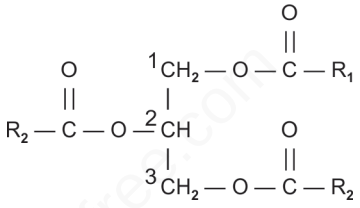


Fig. 6.4: Structure of triacylglycerol

- Main storage form of lipids in the body
- Stored in the adipose tissues
- Otherwise called Neutral fat.

Physical Properties of Triacylglycerol

Saponification and Saponification Number

The hydrolysis of triacylglycerol by alkali into glycerol and soap is called Saponification.

Saponification Number: The mg of KOH required to saponify 1 gm of fat or oil completely. This is a measure of average molecular weight and chain length of the fatty acid present.

- It is inversely proportional to the chain length of fatty acid present in the fats.

Type of fat	Saponification number
Human Fat	195–200
Butter	230–240
Coconut oil	250–260

Iodine number

- The number of grams of Iodine absorbed by 100 g of fat or oil
- Iodine number is used to assess the degree of unsaturation of fat
- It is directly proportional to the degree of unsaturation of fatty acid.

Type of fat/oil	Iodine number
Butter	25–28
Human fat	65–70
Linseed oil	170–200

Reichert-Meissl (RM) Number

The number of 0.1 N KOH required to neutralize the volatile fatty acids distilled from 5 g of fat.

Assess the purity of fats having more volatile fatty acid.

Rancidity of Fat

- Rancidity refers to unpleasant smell and taste for fats and oils
- Hydrolytic rancidity due to partial hydrolysis of Triacylglycerol due to hydrolytic enzymes present in naturally occurring fats and oils
- Oxidative rancidity due to partial oxidation of unsaturated fatty acid
- Vegetable oils with high content of Poly Unsaturated Fatty Acid are easily oxidized. Hence vegetable oils are preserved with antioxidants.

PHOSPHOLIPIDS

Compound lipids composed of fatty acid, alcohol, phosphoric acid and a nitrogenous base. Based on the alcohol present phospholipids are divided into.

- *Glycerophospholipid:* They contain alcohol, Glycerol
- *Sphingophospholipid:* They contain alcohol, Sphingosine.

Glycerophospholipids

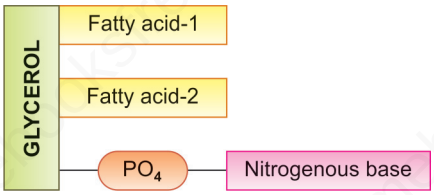


Fig. 6.5: Diagramatic representation of glycerophospholipid

Glycerophospholipid Contain

- Glycerol
- Fatty acid esterified to the first two carbon atoms
- Nitrogenous base
- Phosphoric Acid.

Phosphatidic Acid

Simplest Phospholipid.

Does not contain any nitrogenous base.

Phosphatidic acid contains.

- Glycerol
- Fatty acid esterified to the first two carbon atoms
- Phosphoric Acid.

All glycerophospholipids are derived from Phosphatidic acid.

i.e. Phosphatidic acid + Nitrogenous base

Glycerophospholipids are divided into

Nitrogen Containing

- Lecithin (Phosphatidylcholine)
- Cephalin (Phosphatidylethanolamine)
- Phosphatidyl serine

Nonnitrogen Containing

- Phosphatidylinositol
- Phosphatidylglycerol
- Diphosphatidylglycerol (Cardiolipin)

Lecithin (Phosphatidylcholine)

Glycerophospholipid with Choline as nitrogenous base.

i.e. Phosphatidic Acid + Choline.

Most abundant phospholipid of cell membrane.

Largest body store of choline.

Importance of choline

- Acetylcholine in nerve transmission
- Transmethylation reaction.

Dipalmitoyl Lecithin (Di Palmitoyl Phosphatidyl Choline) is a major constituent of lung surfactant.

Lung Surfactants

Consist of Dipalmitoyl Lecithin, Phosphatidyl Glycerol, Cholesterol and Surfactant protein A, B, C.

Lecithin Sphingomyelin Ratio (L/S ratio)

Before 28 weeks fetal lung synthesizes Sphingomyelin. But as lung matures more Lecithin is synthesized. L/S ratio indicates lung maturity. Ratio of 2 indicate full lung maturity.

Respiratory Distress Syndrome

In premature infants due to decreased lung surfactants.

Cephalin (Phosphatidyl Ethanolamine)

Glycerophospholipid with Ethanolamine as nitrogenous base. i.e. Phosphatidic Acid + Ethanolamine.

- Component of cell membrane
- Play a role in Blood coagulation.

Cardiolipin

- First isolated from Cardiac muscle and hence the name
- Made up of two molecules of Phosphatidic Acid linked by a molecule of Glycerol, i.e. Diphosphatidylglycerol
- It is a major lipid of Inner Mitochondrial Membranes.
- Only Phospholipid which possess antigenicity
- Recognized by antibodies raised against Treponema-pallidum, that causes syphilis
- Decreased Cardiolipin levels or alteration in its structure or metabolism cause mitochondrial dysfunction.

Cardiolipin associated mitochondrial dysfunction is seen in

- Aging
- Heart failure
- Barth Syndrome (Cardioskeletal Myopathy)
- Hypothyroidism

Phosphatidylserine

- Nitrogenous base is Serine
- Plays an important role in Programmed cell death.

Phosphatidylinositol

- Phosphatidylinositol present in the cell membrane
- The Inositol is present as its stereo isomer, Myoinositol
- Play an important role in cell signalling and membrane trafficking
- Phosphatidyl inositol is a precursor of second messenger in hormonal pathways.

Action of Phospholipase C on Phosphatidylinositol 4, 5 Bisphosphate (PiP_2)

- Diacyl Glycerol and Inositol Tris phosphate IP_3 are formed and both act as second messengers.

Phosphatidylinositol 4,5-bisphosphate (PiP_2) —(Phospho Lipase C)→ Diacyl Glycerol + Inositol Tris Phosphate (IP_3)

Ether Lipids

When fatty acid is attached to C1 of Glycerol in Glycerophospholipids by an ether linkage instead of usual Ester linkage, they are called Ether lipids.

Two biologically important Ether Lipids are:

1. Plasmalogen.
2. Platelet Activating Factor (PAF).

Plasmalogen

On C1 instead of fatty acid an unsaturated alkyl is attached by an ether linkage. Components are:

Glycerol + Unsaturated Alkyl residue + Fatty Acid + Phosphoric Acid + Ethanolamine.

- Plasmalogens occur in Brain and heart
- The Plasmalogen may have a protective effect against reactive oxygen species.

Platelet Activating Factor

It has an ether linkage at C1 to an Alkyl residue and Ester linkage at C2 to Acetyl group Components are:

Glycerol + Alkyl residue + Acetic Acid + Phosphoric Acid + Choline.

Sphingophospholipid

The second group of phospholipid which contain **sphingosine** as the backbone alcohol.

Only Sphingophospholipid is Sphingomyelin[Q].

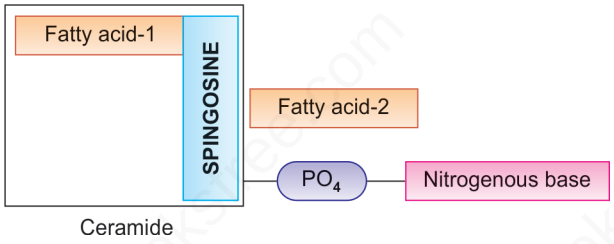


Fig. 6.6: Diagrammatic representation of sphingophospholipids

Sphingomyelin

Structure

- The Amino Alcohol, Sphingosine is attached to a fatty acid by an amide linkage forming Ceramide
- Ceramide is further linked to a phosphoryl and nitrogenous base (Choline) to form Sphingomyelin.

Sphingosine + Fatty Acid = Ceramide
Ceramide + Phosphoryl group + Nitrogenous base = Sphingomyelin

- Sphingomyelins are found in outer leaflet of cell membrane bilayer
- Particularly abundant in specialised areas of plasma membrane called lipid rafts
- They are also particularly abundant in the myelin sheath that surrounds nerve fibers
- They play a role in cell signalling and apoptosis.

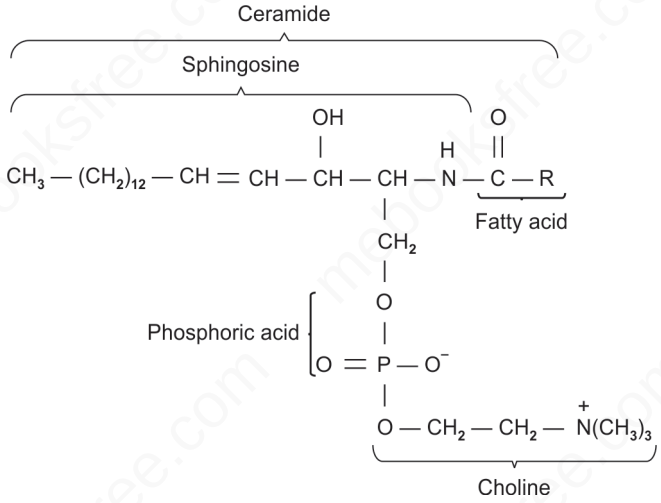


Fig. 6.7: Structure of sphingomyelin

GLYCOLIPIDS (GLYCOSPHINGOLIPIDS)

- Complex lipids which contain carbohydrates, but no phosphoric Acid
- The alcohol in glycolipid is always Sphingosine hence called Glycosphingolipid
- Glycosphingolipids are found in the outer leaflet of Plasma membrane
- Widely distributed in all tissues especially in nervous tissue like brain.

Basic Structure of Glycosphingolipid

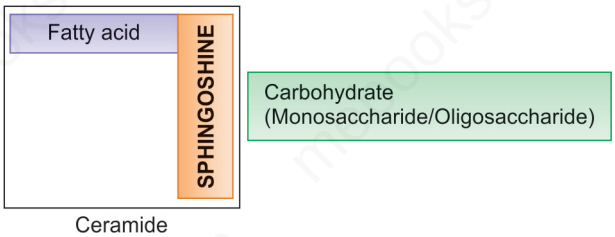


Fig. 6.8: Diagrammatic representation of glycolipids

Three Types Glycolipids

Ceramide (Sphingosine + Fatty Acid) attached to different carbohydrate to form three types of Glycolipids.

Glycolipids or Glycosphingolipids
- Cerebroside
- Globoside
- Ganglioside.

Cerebroside
- Ceramide + Monosaccharide
- Glucocerebroside is Ceramide + Glucose
- Galactocerebroside is Ceramide + Galactose
- Galactocerebroside found in the brain and other neural tissues
- The fatty acid present in Galactocerebroside is characteristically **Cerebronic Acid (C-24)**
- **Sulfogalactosylceramide** present in high amounts in the myelin
- Glucocerebroside found in the **non-neural tissues and some amount in brain.**

Globoside
- Ceramide + Oligosaccharide.

Ganglioside
- Ceramide + Oligosaccharide containing N Acetyl Neuraminic Acid [NANA] or Sialic Acid).

Ganglioside is named as GMn where
- G represent Ganglioside
- M represent Monosialo, as it contain Sialic Acid
- n stands for number assigned on the basis of chromatographic migration. Gangliosides are present in the brain in high concentration.

They act as receptors for bacterial toxins and for hormones

GM3 Ganglioside
- ***The simplest ganglioside found in tissues is GM3***
- **Ceramide + Glucose + galactose + NANA**

GM1 Ganglioside
- More complex than GM3 Ganglioside
- Derived from GM3 Ganglioside
- This is known to be receptor for Cholera toxin in human intestine.

SPHINGOLIPIDOSIS

Sphingolipidoses are a group of lysosomal storage disorder characterized by an inherited deficiency of a lysosomal hydrolase leading to intralysosomal storage of lipid substrates resulting from defective catabolism of the sphingolipids comprising cellular membranes. The lipid substrates share a common structure that includes a ceramide backbone (2-N-acylsphingosine).

General Features of Sphingolipidosis

Progressive lysosomal accumulation of glycosphingolipids in the central nervous system leads to neurodegeneration, storage in visceral cells can lead to organomegaly, skeletal abnormalities, pulmonary infiltration, and other manifestations.

Enzyme deficiencies in sphingolipidosis

Sphingolipidoses	Enzyme deficiency
Farber disease	Ceramidase
Fabry disease	α-galactosidase
GM1 gangliosidosis	β-galactosidase
GM2 gangliosidosis	
1. Tay–Sachs disease,	β-hexosaminidases A
2. Sandhoff disease	β-hexosaminidases A and B
Gaucher's Disease	Glucocerebrosidase/ β Glucosidase
Niemann-Pick Disease	Sphingomyelinase
Metachromatic leukodystrophy	Arylsulfatase A Sphingolipid Activator Protein (SAP-1)
Krabbe's disease	β-galactosidase/ β-galactocerebrosidase

IMPORTANT SPHINGOLIPIDOSIS

GM1 Gangliosidosis

Autosomal recessive trait

Biochemical Defect

- Deficient activity of β-galactosidase, a lysosomal enzyme encoded by a gene on chromosome 3
- Accumulation of GM1 gangliosides in the lysosomes of both neural and visceral cells
- A mucopolysaccharide accumulate in GM1 Gangliosidoses is Keratan Sulfate, in the liver and excreted in urine.

Clinical Presentation

- Most frequently presents in the first 6 months of life with developmental delay followed by progressive psychomotor retardation and the onset of tonic-clonic seizures
- Hepatosplenomegaly, edema, and skin eruptions (**angiokeratoma**)
- **A typical facies** is characterized by low-set ears, frontal bossing, a depressed nasal bridge, and an abnormally long philtrum
- Fifty percent of patients have a **macular cherry red spot**
- By the end of the first year of life, most patients are blind and deaf, with severe neurologic impairment characterized by decerebrate rigidity
- Death usually occurs by 3–4 years of age.

Diagnosis

Demonstration of the deficiency of β-galactosidase activity in peripheral leukocytes.

GM2 Gangliosidoses

This includes:

- Tay–Sachs disease
- **Sandhoff disease.**

Biochemical Defect

- Autosomal Recessive
- Deficiency of β-Hexosaminidase
- Lysosomal accumulation of GM2 Ganglioside, particularly in CNS.

Concept of enzyme defect in GM2 Gangliosidoses

- β-hexosaminidase has two isoforms
- β-hexosaminidase A, composed of 1 α and 1 β subunit
- β-hexosaminidase B, composed of 2 β subunits
- Mutation in α subunit causes Tay–Sachs Disease, so only β-hexosaminidase A
- Mutation in β subunit causes Sandhoffs Disease, so both β-hexosaminidase A and B defect as β subunit is common to both isoforms.

Clinical Features

Tay–Sachs Disease

- The infantile form of Tay–Sachs disease have clinical manifestations in infancy
- Loss of motor skills
- Increased startle reaction (hyperacusis)
- Macular pallor and retinal cherry red spots
- Macrocephaly
- Seizures develop which may be refractory to anticonvulsant therapy
- Neurodegeneration is relentless
- Death occurring by the age of 4 or 5 years.

Sandhoff Disease

Similar to Taysachs but infants with Sandhoff disease have hepatosplenomegaly, cardiac involvement, and mild bony abnormalities.

Diagnosis

Definitive diagnosis is made by determination of β-hexosaminidase A and B activities in peripheral leukocytes.

Gaucher's Disease

Most common Lysosomal Storage Disorder.

Autosomal Recessive.

Biochemical Defect

Deficient activity of the lysosomal hydrolase, acid β-glucosidase (β-glucocerebrosidase). This is encoded by a gene located on chromosome 1q.

The enzymatic defect results in the accumulation of undegraded glycolipid substrates, particularly glucocerebroside (glucosylceramide), in cells of the reticuloendothelial system.

Clinical Manifestations

Type I Gaucher Disease

- Variable age of onset (early childhood to late adult hood)
- Hematological features: Pancytopenia, bleeding manifestation, chronic fatigue
- Hepatosplenomegaly
- Bone Pain (In a pseudo-osteomyelitis pattern) and pathological fractures of long bones
- Bone crises with severe pain and swelling can occur
- No cherry red spot in the macula
- No mental deterioration.

Type 2 Gaucher Disease

- Rapid neurodegenerative course
- With extensive visceral involvement
- Death within the first years of life.

Type 3 Gaucher Disease

- Clinical manifestations are intermediate to those seen in types 1 and 2
- Presents in childhood and death by age 10–15 years.

Diagnosis

- X-ray Femur: Erlenmeyer Flask Deformity
- Bone marrow examination.
  - The pathologic hallmark of Gaucher disease is the Gaucher cell particularly in the bone marrow.

Gaucher cell

- They have characteristic wrinkled paper appearance resulting from the presence of intracytoplasmic substrate inclusions
- The cytoplasm of the Gaucher cell reacts strongly positive with the periodic acid–Schiff stain.

Two other disorders with Gaucher cells

- Granulocytic Leukemia
- Myeloma.

- Determination of the acid β-glucosidase activity in isolated leukocytes or cultured fibroblasts.

Treatment

Enzyme replacement therapy (ERT)

- Mannose terminated Recombinant **Human Acid Beta Glucosidase (Imiglucerase)**
  - Most symptoms including organomegaly, hematological manifestation, bone pain improves.
- Two additional Enzyme Preparation approved by FDA are
  - **Velaglucerase alfa**, which is produced from human fibro sarcoma cells
  - **Taliglucerase alfa**, which is produced in carrot cells.

Oral Substrate reduction agents

- **Miglustat**
  - To decrease glucosylceramide by chemical inhibition of glucosylceramide Synthase.

Bone marrow transplantation

Concept of clinical manifestations and diagnosis of Gaucher Disease

- Accumulation of Glucocerebroside in the reticuloendothelial system, so there is Hepatosplenomegaly
- Infiltration into the bone marrow, so pancytopenia which includes thrombocytopenia, which causes bleeding manifestation and anemia
- Skeletal manifestation like bone pain and pathological fractures.

Diagnosis

- Gaucher disease should be considered in the differential diagnosis of patients with unexplained organomegaly, who bruise easily, have bone pain, or have a combination of these conditions.

Niemann-Pick Disease

Autosomal recessive

Biochemical Defect

- Deficient activity of acid sphingomyelinase, a lysosomal enzyme encoded by a gene on chromosome 11
- Accumulation of sphingomyelin and other lipids in the monocyte-macrophage system.

Clinical Features

- Failure to thrive
- Hepatosplenomegaly
- Rapidly progressive neurodegenerative course.

Treatment

- Orthotopic liver transplantation
- Amniotic cell transplantation
- Bone marrow transplantation
- Miglustat
- A phase I trial of enzyme replacement therapy for type B NPD.

Fabry Disease

X-linked recessive condition.

Biochemical Defect

- Mutations in the α-galactosidase
- A gene located on the long arm of the X. Chromosome (Xq22)
- The enzymatic defect leads to the systemic accumulation of neutral glycosphingolipids, primarily globotriaosylceramide, particularly in the plasma and lysosomes of vascular endothelial and smooth muscle cells.

Clinical Features

- **Angiokeratomas (**telangiectatic skin lesions. Characteristically, the lesions are most dense between the umbilicus and knees, in the 'bathing trunk area,')
- Hypohidrosis
- Corneal and lenticular opacities
- Acroparesthesias
- Pain is the most debilitating symptom in childhood and adolescence
- **Fabry crises**, lasting from minutes to several days, consist of agonizing, burning pain in the hands, feet, and proximal extremities and are usually associated with exercise, fatigue, fever
- Red cell casts and lipid inclusions with characteristic birefringent "Maltese crosses" appear in the urinary sediment.
- Mitral insufficiency is the most common valvular lesion.

Lab Diagnosis

- α-galactosidase A activity in plasma, isolated leukocytes, or cultured fibroblasts or lymphoblasts.

Treatment

Phenytoin and/or carbamazepine

- Decrease the frequency and severity of the chronic acroparesthesias and the periodic crises of excruciating pain.

Renal transplantation and long-term hemodialysis are lifesaving procedures for patients with renal failure.

Enzyme Replacement Therapy

- Recombinant α-galactosidase (Agalzidase β or Fabrazyme) is a safe and effective enzyme replacement therapy of choice for Fabry disease produced from Chinese hamster ovary cells approved by FDA.
- Agalsidase α (Replagal)

Krabbe Disease

- Also called globoid cell leukodystrophy
- Autosomal recessive.

Biochemical Defect

- Deficiency of the enzymatic activity of galactocerebrosidase (Beta Galactosidase)
- Accumulation of galactosylceramide in the white matter of brain
- Galactocerebroside is normally found almost exclusively in the myelin sheath
- The galactocerebrosidase gene is on chromosome 14.

Clinical Features

- The infantile form of Krabbe disease is rapidly progressive and patients present in early infancy with irritability, seizures, and hypertonia
- Optic atrophy is evident in the 1st year of life
- Mental development is severely impaired
- As the disease progresses, optic atrophy and severe developmental delay become apparent; affected children exhibit Opisthotonus and die before 3 years of age.

Diagnosis

Demonstration of the specific enzymatic deficiency in white blood cells or cultured skin fibroblasts.

Farber Disease

Autosomal recessive disorder.

Biochemical Defect

- Deficiency of the lysosomal enzyme acid ceramidase
- The accumulation of ceramide in various tissues, especially the joints.

Clinical Features

- Symptoms can begin as early as the 1st year of life with painful joint swelling and nodule formation over the joints
- Mimicks Rheumatoid Arthritis.

Diagnosis

Ceramidase activity should be determined in cultured skin fibroblasts or peripheral leukocytes.

Wolman Disease and Cholesterol Ester Storage Disease (CESD)

- Autosomal recessive condition
- Lysosomal storage diseases.

Biochemical Defect

- Deficiency of acid lipase
- Accumulation of cholesterol esters and triglycerides in histiocytic foam cells of most visceral organs
- The gene for lysosomal acid lipase is on chromosome 10.

Clinical Features

- Presents in 1st week of life
- Failure to thrive
- Relentless vomiting
- Abdominal distention
- Steatorrhea
- Hepatic dysfunction and fibrosis may occur
- Calcification of the adrenal glands is pathognomonic for the disorder
- Death usually occurs within 6 months.

Cholesterol ester storage disease is a less severe disorder that may not be diagnosed until adulthood.

- Hepatomegaly can be the only detectable abnormality.

Diagnosis

- Measuring acid lipase activity in peripheral leukocytes or cultured skin fibroblasts
- Mutations detection in acid ceramide gene.

Treatment

There is no specific therapy available for either disorder, although pharmacologic agents to suppress cholesterol synthesis, in combination with cholestyramine and diet modification.

Quick review-Sphingolipidoses

Sphingolipidosis with no Cherry red spot on macula-Gauchers Type I, Fabry's Disease

Most Common Lysosomal Storage Disorder- Gaucher Type I

Sphingolipidosis with no mental Deterioration-Gaucher Type I and Fabry's Disease

Sphingolipidosis with X linked recessive inheritance-Fabry's Disease

Contd...

Contd...

Sphingolipidoses with no Hepatosplenomegaly
- Fabry's Disease
- Metachromatic Leukodystrophy
- Krabbe's Disease

Sphingolipidoses with Corneal Clouding
- Fabry's Disease
- GM1 Gangliosidoses

Sphingolipidoses with angiokeratoma
- GM1 Gangliosidoses
- Fabrys Disease

BIOMEMBRANES

Properties of Biomembranes

- Membranes are *sheet like structures,* that form *closed boundaries* between different compartments. The thickness of most membranes is between 60 Å (6 nm) and 100 Å (10 nm)
- Membranes consist mainly of *lipids* and *proteins*. The mass ratio of lipids to proteins ranges from 1:4 to 4:1. Membranes also contain *carbohydrates* that are linked to lipids and proteins
- Membrane lipids are small molecules that have both *hydrophilic* and *hydrophobic* moieties. These lipids spontaneously form *closed bimolecular sheets* in aqueous media. These *lipid bilayers* are barriers to the flow of polar molecules
- *Specific proteins mediate distinctive functions of membranes*. Proteins serve as pumps, channels, receptors, energy transducers, and enzymes
- Membranes are *noncovalent assemblies*. The constituent protein and lipid molecules are held together by many noncovalent interactions, which act cooperatively
- Membranes are *asymmetric*. The two faces of biological membranes always differ from each other.
- Membranes are *fluid structures*
- Lipid molecules diffuse rapidly in the plane of the membrane, as do proteins, unless they are anchored by specific interactions. This is lateral diffusion
- In contrast to lipid molecules, proteins do not rotate across the membrane. The movement of proteins is called transverse diffusion or flip-flop movement.
- Most cell membranes are *electrically polarized,* such that the inside is negative [typically 260 millivolts (mV)]. Membrane potential plays a key role in transport, energy conversion, and excitability.

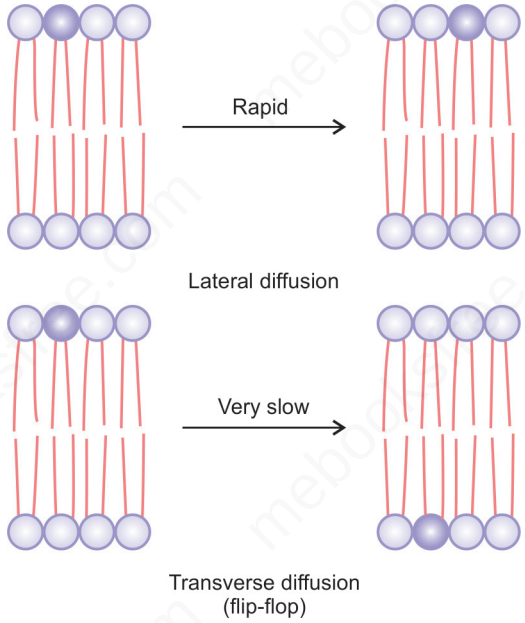


Fig. 6.9: Lateral diffusion and flip-flop diffusion

Components of Membranes

Membranes are complex structures composed of lipids, proteins, and carbohydrate-containing molecules.

Ratio of Protein to Lipid in Different Membranes

Proteins equal or exceed the quantity of lipid in nearly all membranes. The outstanding exception is myelin (Protein to lipid ratio is 0.23).

The Major Lipids in Mammalian Membranes

- Phospholipids
- Glycosphingolipids
- Sterols.

Membrane lipids are amphipathic.

Phospholipid

Two Major Phospholipid Classes Present in Membranes are Glycerophospholipid and Sphingomyelin

- Glycerophospholipid are the most common phospholipid (Lecithin is the most common Glycerophospholipid)
- Choline-containing phospholipids (phosphatidylcholine and sphingomyelin) are located mainly in the outermolecular layer
- Aminophospholipids (phosphatidylserine and phosphatidylethanolamine) are preferentially located in the inner leaflet.

Glycosphingolipids

- Glycosphingolipids are cerebrosides and gangliosides
- The back bone of GSL is ceramide.

Sterol

- The most common sterol in animal cell is cholesterol
- Cholesterol is not present in plants.

Fluid Mosaic Model of Biomembranes[Q]

- Proposed by Singer and Nicolson in 1972
- The membrane consist of bimolecular lipid bilayer with proteins inserted in it or bound to either surface
- This model is likened to integral membrane protein 'icebergs' floating in a sea of predominantly fluid phospholipid molecules.

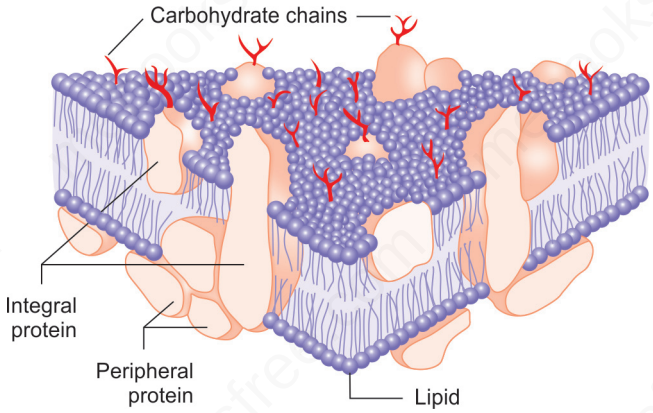


Fig. 6.10: Fluid mosaic model of plasma membrane

Membrane Proteins

Membranes Contain Integral and Peripheral Proteins.

Integral Protein

- Deeply embedded (to both hydrophilic and hydrophobic portions) in the lipid bilayer
- Usually globular and are themselves amphipathic
- Trans membrane integral protein are the proteins that span the whole lipid bilayer.

Peripheral Proteins

- Attached to the Hydrophilic portions of Plasma membrane

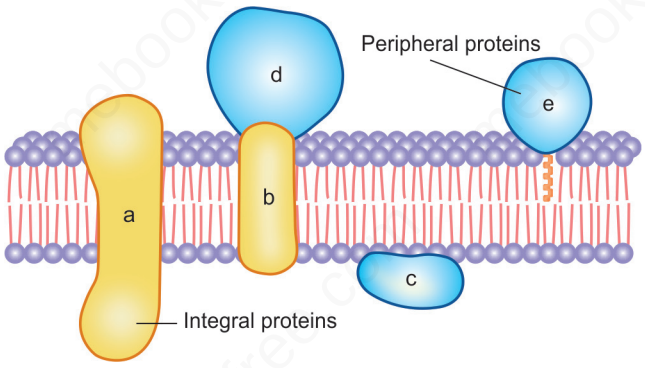


Fig. 6.11: Peripheral proteins and integral proteins

- Do not interact directly with the hydrophobic cores of the phospholipids in the bilayer.

Fluidity of Membranes

The temperature at which the structure undergoes the transition from ordered to disordered (i.e. melts) is called the 'transition temperature' (Tm).

Factors Affecting the Melting Temperature

The properties of fatty acids and lipids derived from them are markedly dependent on chain length and degree of saturation.

- The longer and more saturated fatty acid chains cause higher values of Tm. So decreases the fluidity of membranes
- Unsaturated fatty acid increases the fluidity (i.e. they lower Tm)
- Cholesterol modifies the fluidity of membranes.
  - At temperatures below the Tm increases fluidity
  - At temperatures above the Tm decreases fluidty.

At normal body temperature (37°C) the lipid bilayer is in a fluid state.

SPECIALIZED REGION OF PLASMA MEMBRANE

Lipid Rafts[Q]

Lipid rafts are specialized areas of the exoplasmic leaflet of the lipid bilayer: Enriched **in cholesterol, sphingolipids.** Contain **certain GPI-linked proteins (outer leaflet)** and acylated and prenylated proteins (inner leaflet). Important for signal transduction and other processes.

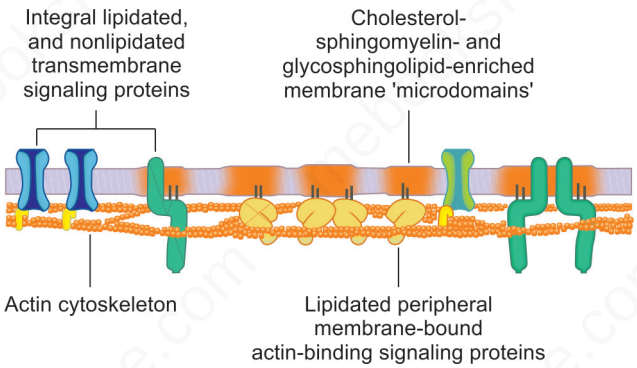


Fig. 6.12: Lipid rafts

Caveolae

Flask shaped indentation of the cell membrane facing the cytosol. Contain the protein caveolin-1.

Functions

- Signal transduction system (e.g. the insulin receptor and some G proteins), the folate receptor, and endothelial nitric oxide synthase (eNOS)
- Transport of macromolecules [IgA]
- Endocytosis of Cholesterol containing lipoprotein.

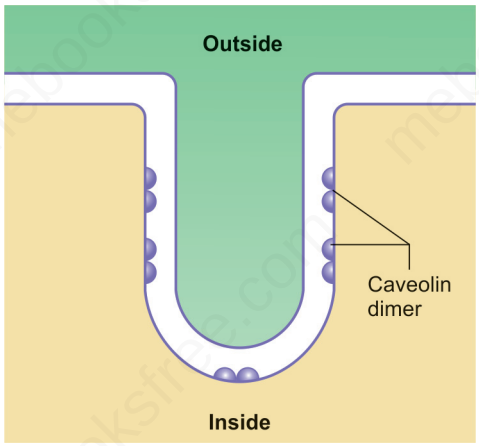


Fig. 6.13: Caveolae

Intercellular Connections

Intercellular junctions that form between the cells in tissues can be broadly split into two groups:

- Junctions that fasten the cells to one another and to surrounding tissues
- Junctions that permit transfer of ions and other molecules from one cell to another.

Group I: The Types of Junctions That Tie Cells Together

- Tight junctions [the zonulaoccludens]
  - Prevent the diffusion of macromolecules between cells.
  - Are composed of various proteins, including occludin, various claudins, and junctional adhesion molecules [JAMs]
  - Absence of Tight junction implicated in loss of contact inhibition
- Desmosome and zonula adherens also help to hold cells together

- Hemidesmosome and focal adhesions attach cells to their basal laminas.

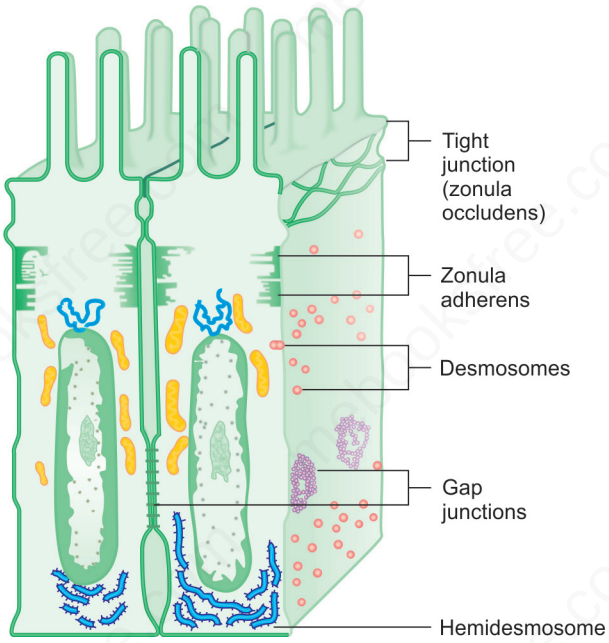
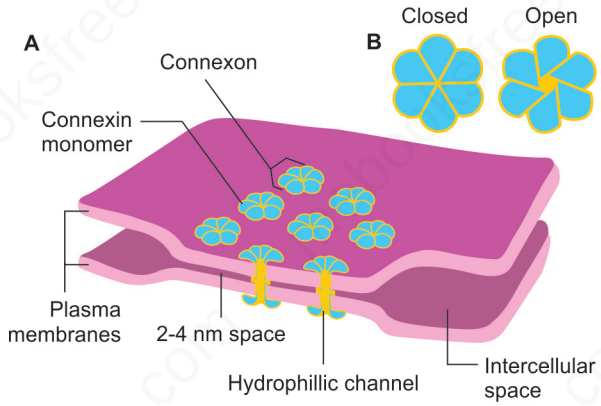


Fig. 6.14: Intercellular connections

Group II: Junctions That Permit Transfer of Ions and Other Molecules from One Cell to Another

Gap Junctions

- Cytoplasmic 'tunnel' for diffusion of small molecules (< 1000 Da) between two neighbouring cells
- At gap junctions, the intercellular space narrows from 25 to 3 nm, and units called connexons in the membrane of each cell are lined up with one another
- Each connexon is made up of six protein subunits called connexins.



Figs 6.15A and B: Gap junction

Self-oriented structures formed by amphipathic lipids

- Amphipathic lipids self-orient at Oil: Water interfaces
- They form Membranes, Micelles, Liposomes, and Emulsions.

Lipid bilayer

A bilayer of such amphipathic lipids is the basic structure in biologic **membranes.**

Micelle

When a critical concentration of these lipids is present in an aqueous medium, they form **micelles**. Aggregation of bile salts into micelles and liposomes and the formation of **mixed micelles** with the products of fat digestion are important in facilitating absorption of lipids from the intestine.

Liposomes

- Are formed by sonicating an amphipathic lipid in an aqueous medium
- They consist of spheres of lipid bilayers that enclose part of the aqueous medium.

Clinical uses of liposomes

- As carriers of drugs in the circulation, targeted to specific organs, for example, in cancer therapy
- They are used for gene transfer into vascular cells as carriers for topical and transdermal delivery of drugs and cosmetics.

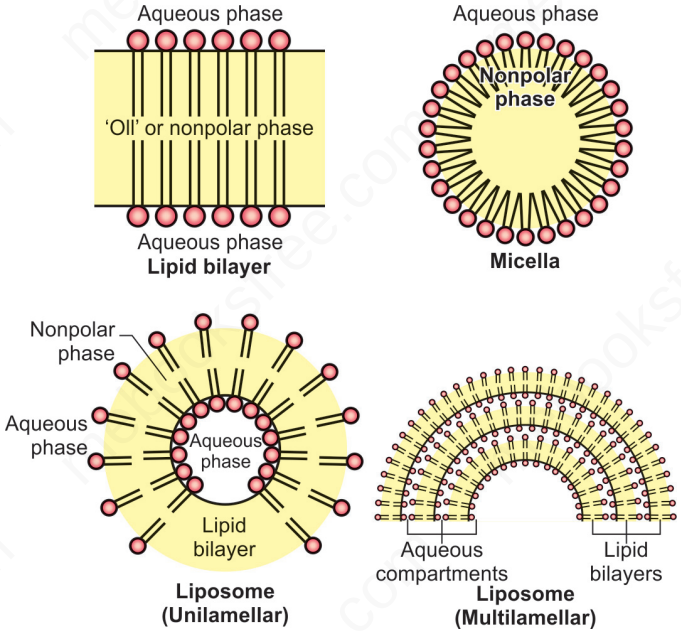


Fig. 6.16: Self-oriented structures formed by amphipathic lipids

REVIEW QUESTIONS

Lipids of Physiological Significance

1. Essential fatty acid is/are: (PGI May 2013)

a. Palmitic acid
b. Linoleic acid
c. Linolenic acid
d. Oleic acid
e. Free fatty acid

Ans. b. Linoleic acid, **c.** Linolenic Acid.

Essential fatty acid

The fatty acids that are required by humans, but are not synthesized in the body hence need to be supplied in the diet are known as essential fatty acid (EFA). Humans lack the enzymes that can introduce double bond beyond 9th Carbon.

They are polyunsaturated fatty acid namely

- **Linoleic Acid**
- **Linolenic Acid**

Linoleic acid is the most essential fatty acid ***arachidonic Acid*** is considered as ***Semi essential fatty Acid*** as it can be synthesized from Linoleic Acid.

Functions of essential fatty acid

- They are integral components of membrane structure, often in the 2 position of phospholipids
- Eicasanoids are synthesized from Arachidonic Acid. Essential fatty acids are needed for the synthesis of Arachidonic Acid
- Lower the risk of Cardiovascular Diseases.

2. True statement about fatty acid: (PGI Nov 2011)

a. PUFA is essential for membrane structure
b. Biologically arachidonic acid is essential to life
c. Hydrogenated vegetable oils contains trans fatty acid
d. Most of the naturally occurring unsaturated FA exist as trans isomer

Ans. a, b, c. *(Ref: Harper 30/e p241)*

Most of the naturally occurring UFA exist in cis form.

3. True about trans fatty acid: (PGI Nov 2010)

a. Fried rice have high content of Trans Fatty Acid
b. Partial Hydrogenation increases Trans Fatty Acid
c. Refining decreased TFA
d. ↑ LDL
e. ↑ HDL

Ans. a, b, d, e. *(Ref: Harper 30/e p241)*

Trans fatty acids

- Present in dairy products and **partially hydrogenated** edible oils
- They are used in food industry to improve the shelf life
- Trans fatty acids are present in high amounts in processed foods, fast foods and bakery items
- Consumption of trans fatty acid for long-terms may raise the risk factor for cardiovascular diseases like Atherosclerosis and Coronary Artery disease and Diabetes mellitus
- Exacerbate essential fatty acid deficiency by competing for essential fatty acid.

4. PUFA (more than 50%) content is seen in: (PGI Nov 2009)

a. Groundnut oil
b. Safflower oil
c. Corn oil
d. Sunflower oil

Ans. b, c, d. *(Ref: Park 23/e p611 Table 2)*

- Highest content of MUFA in Mustard/Rapeseed oil
- Highest content of Medium chain fatty acid in Coconut oil
- Highest content Linoleic acid in Safflower oil
- Highest content alpha linolenic acid in Flax seed oil

Fats/Oil	SFA (%)	MUFA (%)	Linoleic acid (%)	Alpha linolenic acid (%)
High Medium chain and Short chain fatty acid				
Coconut	92	6	2	–
Palm kernel	83	15	2	–
Butter/Ghee	68	29	2	1
High SFA and MUFA				
Palmolein	39	46	11	< 0.5
High MUFA and Moderate Linoleic acid				
Ground nut	19	41	32	< 0.5
Rice bran	17	43	38	1
Sesame	16	41	42	< 0.5

Contd...

Contd...

Fats/Oil	SFA (%)	MUFA (%)	Linoleic acid (%)	Alpha linolenic acid (%)
High linoleic acid				
Cotton seed	24	29	48	1
Corn	12	35	**50**	1
Safflower	9	13	**75**	–
Sunflower	12	22	**62**	–
High Linoleic acid and alpha linolenic acid				
Soyabean	14	24	**53**	7
Canola	6	60	22	10
Mustard/Rape-seed	4	65	15	14
Flax-Seed	10	21	16	5
High trans fatty acid				
Vanspati	46	49	4	–

5. Which among the following is a cardio protective fatty acid? (Kerala 2011)

a. Palmitic acid
b. Stearic acid
c. Oleic acid
d. Omega-3 fatty acid

Ans. d. Omega-3 fatty acid. *(Ref: Harper 30/e p248)*

Significance of ω3 Fatty Acid

- Decrease the risk of Cardiovascular Disease
- Appear to replace arachidonic acid in platelet membranes
- Lower the production of thromboxane and tendency of the platelet aggregation
- Decrease Serum Triglycerides
- Important for Infant Development
- Lower the risk of various mental illness (Depression, ADHD)
- Lower the risk of chronic degenerative diseases such as Cancer, Rheumatoid Arthritis, and Alzheimers Disease.

Omega Classification of Fatty Acids	
ω3 Series	***α Linolenic Acid*** ***Timnodonic Acid (EicosaPentaenoic Acid)*** ***Cervonic Acid (DocosaHexaenoic Acid) (DHA)***
ω6 Series	***Linoleic Acid*** ***γLinolenic Acid (GLA) Arachidonic Acid***
ω9 Series	***Oleic Acid Elaidic Acid***

6. Which among the following is not a saturated fatty acid? (Kerala 2009)

a. Myristic acid
b. Stearic acid
c. Palmitic
d. Linoleic acid

Ans. d. Linoleic acid. *(Ref: Harper 30/e p213)*

Common Saturated Fatty Acids and Their Sources

Saturated fatty acid	Source
Acetic Acid (2C)	Vinegar
Butyric Acid (4C)	Butter
Valeric Acid (5C)	Butter
Caproic Acid (6C)	Butter and Coconut milk
Lauric Acid (12C)	Coconut milk
Myristic Acid (14C)	Coconut milk
Palmitic Acid (16C)	Body Fat
Stearic Acid (18C)	Body Fat

7. Most essential fatty acid is: (Kerala 2008)

a. Linolenic acid
b. Linoleic acid
c. Arachidonic acid
d. Eicosapentaenoic acid

Ans. b. Linoleic Acid. *(Ref: Harper 30/e p 222, 241)*

- Linoleic Acid is the Most Essential Fatty Acid

Arachidonic Acid is considered as ***Semi essential fatty Acid*** as it can be synthesized from Linoleic Acid.

Functions of essential fatty acid

- They are integral components of membrane structure, often in the 2 position of phospholipids
- Eicasanoids are synthesized from Arachidonic Acid. Essential fatty acids are needed for the synthesis of Arachidonic Acid
- Lower the risk of Cardiovascular Diseases.

8. All are true except: (PGI Dec 2002)

a. Linoleic acid is found in Soyabean oil
b. Linolenic and linoleic acids are cis derivatives containing double bonds
c. Arachidonic acid contain five double bonds
d. Monoenoic acids contain one double bond at 9th position

Ans. c. Arachidonic acid contain five double bonds.

(Ref: Harper 30/e p212, 238)

- Fifty-three percent of Soyabean oil is Linoleic acid
- Almost all unsaturated fatty acids have cis configuration
- Arachidonic acid has 4 double bonds
- First double bond is usually added in 9th position by a delta 9 desaturase.

9. Maximum source of linoleic acid is: **(AIIMS Jun 97)**

a. Coconut oil
b. Sunflower oil
c. Palm oil
d. Vanaspati

Ans. b. Sunflower oil.

- Highest content of MUFA in Mustard/Rapeseed oil
- Highest content of Medium chain fatty acid in Coconut oil
- Highest content Linoleic acid in Safflower oil
- Highest content alpha linolenic acid in Flax seed oil.

10. Which of these fatty acids is found exclusively in breast milk: **(AI 2001)**

a. Linolaete
b. Linolenic
c. Palmitic
d. Docosahexanoic acid

Ans. d. Docosa Hexaenoic acid.

(Ref: Harper 30/e p214 Table 22)

- Docosahexaenoic acid is present in fish oils, phospholipids of brain, algal oils
- DHA is otherwise called Cervonic acid
- It belongs to ω3 fatty acid.

11. The following fatty acid does not belong to ω6 series linoleic acid: **(PGI June 2000, AIIMS Dec 90)**

a. Arachidonic acid
b. Gamma-linoleic acid
c. Alpha-linolenic acid
d. Timnodonic acid

Ans. d. Alpha linolenic acid. *(Ref: Harper 30/e p214)*

ω3 FA	ω6 FA	ω7 FA	ω9 FA
Alpha-linolenic acid	Linoleic acid	Palmitoleic acid	Oleic acid
Timnodonic acid (Eicosapentaenoic acid)	Gamma-linolenic acid		
Cervonic Acid (Docosahexaenoic acid)	Arachidonic acid		

12. An example Omega 6 Fatty acid is: **(CMC Ludhiana 2014)**

a. Cervonic Acid
b. αLinolenic Acid
c. Arachidonic Acid
d. Timnodonic acid

Ans. c. Arachidonic Acid. *(Ref: Harper 30/e p214)*

13. Which is not present in plants? **(CMC Ludhiana 2014)**

a. Cholesterol
b. Linolenic acid
c. Linoleic Acid
d. Lauric Acid

Ans. a. Cholesterol.

Phospholipids and Glycolipids

14. Gangliosides contain: **(PGI May 2015)**

a. Phosphate
b. Galactose
c. Sulfate
d. Serine
e. Sialic Acid

Ans. b. Galactose, **e.** Sialic Acid.

(Ref: Harper 30/e p218)

Ganglioside

- Contain Sphingosine + Fatty acid + Oligosaccharide that contain one or two molecules of sialic acid.

15. Glycosphingolipid is made up of: **(PGI Nov 2010)**

a. Glucose
b. Glycerol
c. Sphingosine
d. Fatty acids
e. Thromboxane A2

Ans. a, c, d. *(Ref: Harper 30/e p219)*

Phospholipids

Lipids containing phosphoric acid and nitrogenous base in addition to glycerol and fatty acid. Fatty Acid + Alcohol (Glycerol/Sphingosine) + Phosphate + Nitrogenous base

Glycerophospholipids

The alcohol in this group is Glycerol, e.g. Lecithin, Cephalin.

Sphingophospholipids

The alcohol in this group is Sphingosine, e.g. Sphingomyelin.

Glycolipids or Glycosphingolipids

Lipids containing carbohydrate apart from Fatty acid and Alcohol (usually Sphingosine).

Fatty Acid + Alcohol (Sphingosine) + Carbohydrate, e.g. Cerebroside, Ganglioside.

16. Alcoholic group is found in: (PGI June 98)

a. Ganglioside
b. Sphingomyelin
c. Cerebroside
d. Ceramide

Ans. a, b, c, d.

- Ceramide = Sphingosine (Amino alcohol) + Fatty acid
- Ganglioside = Ceramide + Oligosaccharide that contains N Acetyl Neuraminic acid
- Cerebroside = Ceramide +Monosaccharide
- Sphingomyelin = Glycerol + 2 Fatty acid + PO_4 + Choline.

17. Which of the following is a glycolipid? (JIPMER 2012)

a. Cerebroside
b. Plasmalogen
c. Sphingomyelin
d. Lecithin

Ans. a. Cerebroside. *(Ref: Harper 30/e p218)*

Glycolipids or Glycosphingolipids

- Cerebroside
- Globoside
- Ganglioside.

18. Second messenger is produced from:

a. Phosphatidylinositol
b. Phosphatidylserine
c. Phosphatidylcholine
d. None

Ans. a. Phosphatidylinositol. *(Ref: Harper 30/e p216)*

Phosphatidylinositol

- Phosphatidyl Inositol present in the cell membrane
- The inositol is present as its stereo isomer, Myoinositol.
- Play an important role in cell signalling and membrane trafficking
- Phosphatidylinositol is a precursor of second messenger in hormonal pathways.

Action of Phospholipase C on Phosphatidylinositol 4,5 Bisphosphate (PiP_2)

Diacylglycerol and Inositol Tris phosphate IP3 are formed and both act as second messengers.

Sphingolipidosis

19. A child presents with hepatosplenomegaly and pancytopenia. Bone marrow shows 'crumbled tissue paper appearance'. It is due to accumulation of: (AIIMS May 2013)

a. Glucocerebroside
b. Sphingomyelin
c. Ganglioside
d. Galactocerebroside

Ans. a. Glucocerebroside.

(Ref: Nelson 20/e Chapter 86 4 Lipidoses)

Sphingolipidoses	Enzyme Deficiency	Sphingolipid accumulated
Farber disease	Ceramidase	Ceramide
Fabry disease	α-galactosidase	Globotriosylceramide
GM1 gangliosidosis	β-galactosidase	GM1 ganglioside
GM2 Gangliosidosis		
Tay-Sachs disease,	β-hexosaminidases A	GM2 ganglioside
Sandhoff disease	β-hexosaminidases A and B	GM2 Ganglioside
Gaucher disease	Glucocerebrosidase/ β-glucosidase	Glucosylceramide
Niemann-Pick Disease	Sphingomyelinase	Sphingomyelin
Metachromatic leukodystrophy	Arylsulfatase A Sphingolipid Activator Protein (SAP-1)	Sulfogalactosylceramide
Krabbe disease	β-galactosidase β-galactocerebrosidase	Galactosylceramide

Gaucher's Disease

- Most common Lysosomal Storage Disorder
- Glucocerebrosidase defect
- Lysosomes filled with Glucocerebroside
- No cherry red spot in the macula
- No mental deterioration (Type I)
- Hematological features: Pancytopenia, bleeding manifestation
- Hepatosplenomegaly
- Bone Pain and Pathological Fractures of long bones
- X-ray Femur Erlenmeyer Flask Deformity
- Bone Marrow Biopsy Gaucher cell with Wrinkled paper appearance/crumbled tissue paper appearance.

20. Which is/are sphingolipidosis? (PGI May 2013)

a. Tay-Sachs disease
b. Fabry's disease

c. Krabbe's disease
d. Sandhoff's disease
e. Wolman's disease

Ans. a, b, c, d. *(Ref: Nelson 20/e Chapter 86.4 Lipidoses)*

Sphingolipidosis	Enzyme defect
Fabry disease	α-galactosidase
Farber disease	Ceramidase
Galactosialidosis	β-galactosidase and sialidase
GM1 gangliosidosis	β-galactosidase
GM2 gangliosidosis	
Tay-Sachs disease	β-hexosaminidases A
Sandhoff's disease	β-hexosaminidases A and B
Gaucher Type I	Glucocerebrosidase
Gaucher Type II	Glucocerebrosidase
Gaucher Type III	Glucocerebrosidase
Niemann-Pick Type A	Sphingomyelinase
Niemann-Pick Type B	Sphingomyelinase
Metachromatic leukodystrophy	Arylsulfatase A or Sphingolipid Activator Protein (SAP-1)
Krabbe disease	β-galactocerebrosidase /β-galactosidase

21. Sphingomyelinase Deficiency is seen in: (AI 2010)

a. Niemann-Pick disease
b. Farber's disease
c. Tay-Sachs disease
d. Krabbe's disease

Ans. a. Niemann-Pick disease.
(Ref: Nelson 20/e Chapter 86.4 Lipidoses)

Other options
- Farber's Disease—Ceramidase
- Tay-Sachs Disease-β-hexosaminidase A
- Krabbe's Disease-β-galactosidase.

22. Deficiency of phosphorylating enzymes for the formation of which of the following recognitior marker leads to I- cell disease? (Kerala 2008)

a. GM2 ganglioside
b. Mannose 6-phosphate
c. Galactose
d. Globoside

Ans. b. Mannose 6-phosphate. *(Ref: Harper 30/e p586)*
Disorders associated with defect in import of Proteins to Lysosomes.

I cell Disease
- Due to defective synthesis of recognition marker – Mannose 6-phosphate
- Serum level of Lysosomal enzymes raised.

23. Which of the following disease occurs due to the deficiency of glucocerebrosidase? (Kerala 2008)

a. Gaucher disease
b. Pompe disease
c. Fabry disease
d. Krabbe disease

Ans. a. Gaucher Disease.
(Ref: Nelson 20/e Chapter 86.4 Lipidoses)

24. Accumulation of sphingomyelin in phagocytic cells is feature of: (AIIMS Nov 02)

a. Tay–sachs disease
b. Gaucher's disease
c. Niemann-Pick disease
d. Down's syndrome

Ans. c. Niemann-Pick Disease.

Niemann-Pick Disease Autosomal Recessive **Biochemical defect**
- Deficient activity of acid sphingomyelinase, a lysosomal enzyme encoded by a gene on chromosome 11
- Accumulation of sphingomyelin and other lipids in the monocyte-macrophage system.

Clinical features
- Failure to thrive
- Hepatosplenomegaly
- Rapidly progressive neurodegenerative course.

Treatment
- Orthotopic liver transplantation
- Amniotic cell transplantation
- Bone marrow transplantation
- Miglustat
- A phase I trial of enzyme replacement therapy for type B NPD.

25. Tay-Sachs disease is due to accumulation of: (JIPMER 2012)

a. GM2 ganglioside
b. GM1 ganglioside
c. Glucocerebroside
d. Galactocerebroside

Ans. a. GM2 gangliosidoses.
(Ref: Nelson 20/e Chapter 86.4 Lipidoses)

Biomembranes

26. Eukaryotic plasma membrane is made up of all except: (May 2009)

a. Carbohydrates
b. Triglycerides
c. Lecithin
d. Cholesterol

Ans. b. Triglycerides *(Ref: Harper 30/e p478)*

- Membranes are Complex Structures Composed of Lipids, Proteins, and Carbohydrate-Containing Molecules
- The Major Lipids in Mammalian Membranes Are Phospholipids, Glycosphingolipids and Cholesterol
- Two major phospholipid classes present in membranes, **phosphoglycerides** are the more common
- Among the Phosphoglycerides, lecithin is the most abundant.

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7 Metabolism of Lipids

Topics Included

- Digestion and Absorption of Lipids
- Metabolism of Simple Lipids
  - Triacylglycerol
- Metabolism of Compound Lipids
  - Phospholipids
  - Glycolipids
- Metabolism of Fatty Acids
- Metabolism of Ketone Bodies
- Metabolism of Cholesterol
- Metabolism of Lipoproteins

DIGESTION AND ABSORPTION OF LIPIDS

The major lipids in the diet are triacylglycerols (>90%) and the rest is made of phospholipids, cholesterol, cholesterol esters and free fatty acids.

Enzymes for Digestion of Lipids

Lingual lipase and gastric lipase

- Hydrolysis of triacylglycerols is initiated by lingual and gastric lipases
- Site: Stomach
- Action: Hydrolysis of 3rd ester bond of Triacylglycerol forming 1,2 Diacylglycerol.
- 30% of total Triacylglycerol digestion takes place in the stomach.

Pancreatic lipase

- Requires a further pancreatic protein, colipase, for activity.
- Colipases prevents the inhibition Lipases by bile acids.
- This is the major enzyme of Triacylglycerol hydrolysis
- It is specific for the primary ester links, i.e. positions 1 and 3 in triacylglycerol
- Resulting in 2-monoacylglycerols and free fatty acids
- 2-Mono Acylglycerol and fatty acids are the major end products of luminal triacylglycerol digestion.

Pancreatic esterase

- 25% of monoacylglycerols are hydrolyzed to glycerol and fatty acids.
- Cholesterol esters and other lipid esters are also hydrolized to some extent.

Phospholipases

- Specifically Phospholipases A2 is present in Pancreatic juice.

Absorption of Lipids

Role of Bile Salt/Bile Acids

- Synthesized in the liver.
- They are biological detergents.
- At physiological pH bile acids are seen ionized (anions) so bile acids and bile salts (anions) are interchangeably used.
- The products of lipid digestion are hydrophobic molecules.
- Bile salt/Bile acids helps in emulsification of products of lipid digestion to micelle.

- The minimal concentration of bile acids necessary for micelle formation is called Critical Micellar Concentration. It is about 5 mm.
- Micelles are less than 1 μm in diameter and are soluble.
- They allow the products of digestion, to be transported through the aqueous environment of the intestinal lumen to come into close contact with the brush border of the mucosal cells, allowing uptake into the epithelial cells.
- The fat-soluble vitamins, A, D, E and K also transported in the micelles.

Within the Intestinal Epithelial Cells

- 1-monoacyglycerols are hydrolyzed to fatty acids and glycerol.
- Short chain fatty acid and medium chain fatty acids are passed into portal vein without any modification.
- Glycerol released in the intestinal lumen is absorbed into the hepatic portal vein.
- Long chain fatty acid and 2-monoacylglycerol are transported into endoplasmic reticulum.
- Long-chain fatty acids and 2-monoacylglycerols are reacylated to triacylglycerols via the monoacylglycerol pathway.
- Cholesterol is esterified by Cholesterol Acyltransferase
- The newly synthesized Triacylglycerol, Cholesterol ester and phospholipids are incorporated into Chylomicrons.
- Chylomicrons are secreted into the lymphatics, enter blood stream via thoracic duct.

METABOLISM OF TRIACYLGLYCEROL

Synthesis of Triacylglycerol

Triacylglycerol are the predominant form of simple lipids, which are composed of Glycerol esterified to three Fatty acids.

Site: Almost all tissues but predominantly in Liver and Adipose tissue, Intestinal mucosal cells.

Orgenelle: Majority Endoplasmic Reticulum, a few in mitochondria.

Three steps for TAG synthesis

1. Activation of Fatty Acid.
2. Activation of Glycerol.
3. Esterification of fatty Acid to Glycerol.

Activation of Fatty Acid

- **Enzyme:** Acyl CoA Synthetase or Thiokinase
- **Reaction:** Fatty acid converted to CoA derivative.

Activation of Glycerol

- **Enzyme:** Glycerol Kinase
- **Reaction:** Glycerol is phosphorylated to Glycerol 3 phosphate.

In Muscle and Adipose Tissue

Glycerol Kinase is absent in muscle and white adipose tissue.

Glycerol 3 Phosphate is formed from Dihydroxy Acetone Phosphate, an intermediate in Glycolysis.

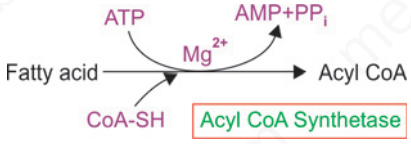


Fig. 7.1: Activation of fatty acid

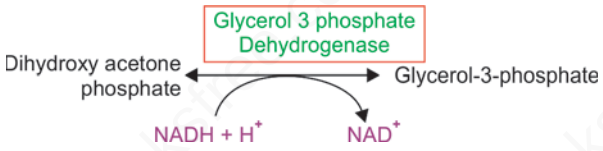


Fig. 7.2: Activation of glycerol

Esterification of Activated Fatty Acid to Triacylglycerol

- **Enzyme:** Acyltransferase
- **Reaction:** Transfer of 3 Acyl moiety to Glycerol successively.

Synthesis of Triacylglycerol in the Intestine-Monoacylglycerol Pathway

In the **intestinal Mucosa**, Monoacylglycerol Transferase converts Monoacylglycerol to 1,2 Diacylglycerol in the **Monoacylglycerol Pathway**.

Degradation of Triacylglycerol (Lipolysis)

- Triacylglycerols are hydrolyzed by a **lipase** to their constituent fatty acids and glycerol
- Much of this hydrolysis (lipolysis) occurs in adipose tissue.
- The free fatty acids is released into the plasma, where they are found combined with serum albumin.
- The free fatty acid uptake into tissues (including liver, heart, kidney, muscle, lung, testis, and adipose tissue, but not readily by brain), where they are oxidized or re-esterified.

- The utilization of glycerol depends upon whether such tissues have the enzyme **glycerol kinase**.
- The Glycerol kinase is found in significant amounts in liver, kidney, intestine, brown adipose tissue, and the lactating mammary gland.

NB: Brown Adipose Tissue contains Glycerol Kinase unlike White Adipose Tissue which lack Glycerol Kinase.

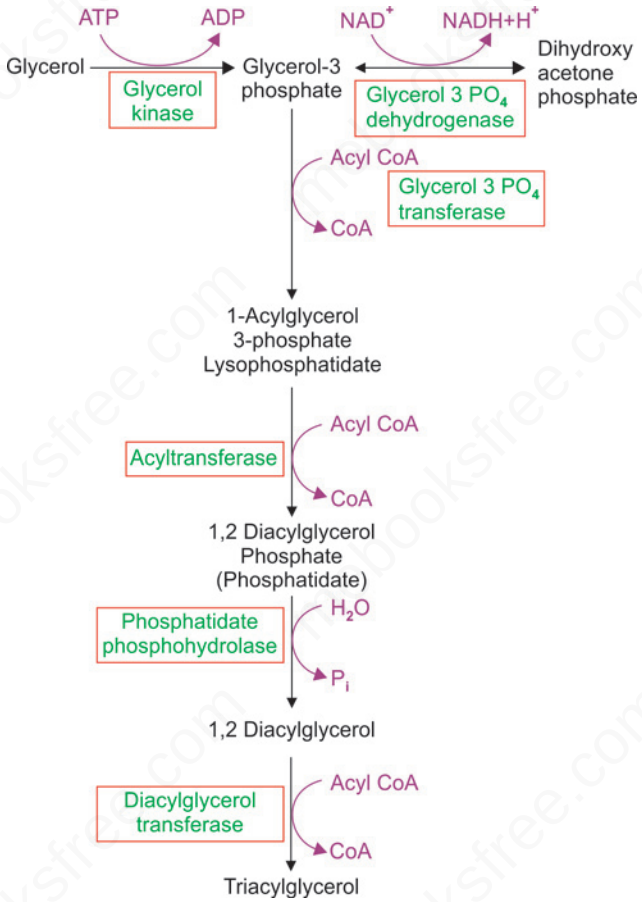


Fig. 7.3: Synthesis of triacylglycerol

LIPOLYSIS IN ADIPOSE TISSUE

- Stored fat, Triacylglycerol (TAG) is degraded.
- By special enzyme: hormone sensitive Lipase (hSL)

Reaction: Hormone sensitive Lipase removes Fatty acid from 1st and 3rd Carbon of TAG to form Diacylglycerol, and Monoacylglycerol sequentially.

The Hormone sensitive Lipase cannot remove Fatty acid from 2nd Carbon atom which is removed by 2-Monoacylglycerol Lipase.

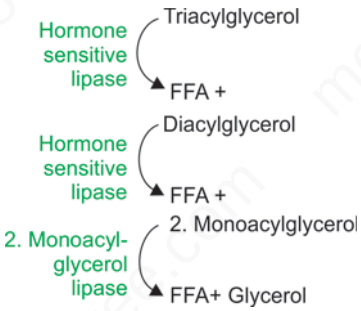


Fig. 7.4: Action of hormone sensitive lipase

Regulation of Hormone Sensitive Lipase

- The activity of this enzyme is under the control of hormones, hence the name.
- It is present in the adipose tissue.
- It is active in phosphorylated state
- It is inactive in dephosphorylated state.

Hormone Sensitive lipase is activated by:

- Glucagon
- Catecholamines (Epinephrine and Norepinephrine)
- ACTH
- TSH
- Glucocorticoids
- Thyroid hormones
- Growth hormone
- α and β Melanocyte Stimulating Hormone (MSH)
- Vasopressin.

Mechanism of activation of Hormone Sensitive LipaseQ

- By stimulating the activity of **adenylyl cyclase,** the enzyme that converts ATP to cAMP.
- cAMP, by stimulating **cAMP-dependent protein kinase,** activates hormone-sensitive lipase by phosphorylation.
- But Glucocorticoids promote lipolysis via synthesis of new lipase protein by a cAMP-independent pathway.

Hormone sensitive Lipase is inactivated by

- Insulin
- Nicotinic Acid
- Prostaglandin E1

Mechanism of Inactivation of Hormone Sensitive Lipase

The antilipolytic effects of insulin, nicotinic acid, and prostaglandin E1 are accounted for by inhibition of the synthesis of cAMP at the adenylyl cyclase site, acting through a Gi protein.

Insulin also stimulates phosphodiesterase inactivates hormone-sensitive lipase. See Figure 7.5 for regulation of hormone sensitive lipase.

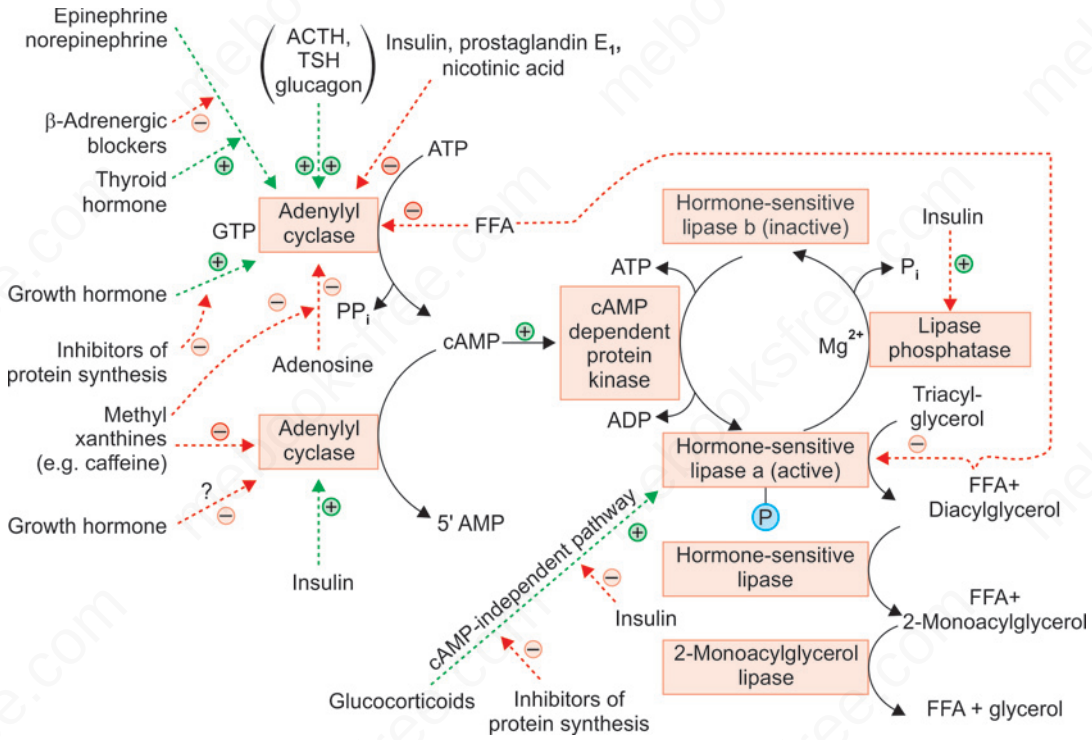


Fig. 7.5: Regulation of hormone sensitive lipase

METABOLISM OF FATTY ACIDS

- Fatty acid synthesis
- Fatty acid oxidation
- Ketone body metabolism

De Novo Fatty Acid Synthesis (Lipogenesis)

Major contribution to discovery by Feodor Lynen, hence the pathway is otherwise called **Lynen's Spiral**.

Site^{Q}: Liver, kidney, brain, lung, lactating mammary gland, and adipose tissue.

Organelle:^{Q} By an extramitochondrial system [in the cytosol].

Cofactor^{Q} requirements include NADPH, ATP, Mn^{2+}, Biotin, and HCO^{3-} (as a source of CO_2)

Acetyl-CoA^{Q} is the principal building block of Fatty Acid.

Sources of Acetyl CoA

- Aerobic Glycolysis [Pyruvate to Acetyl CoA by Pyruvate Dehydrogenase (PDH) in the mitochondria]
- Fatty Acid Oxidation (in the mitochondria).

Transport of Acetyl CoA

- Formation of Acetyl CoA by the above processes occur in the mitochondria.
- Fatty acid Synthesis takes place in the Cytosol.
- Acetyl CoA is not readily diffused through Mitochondrial membrane

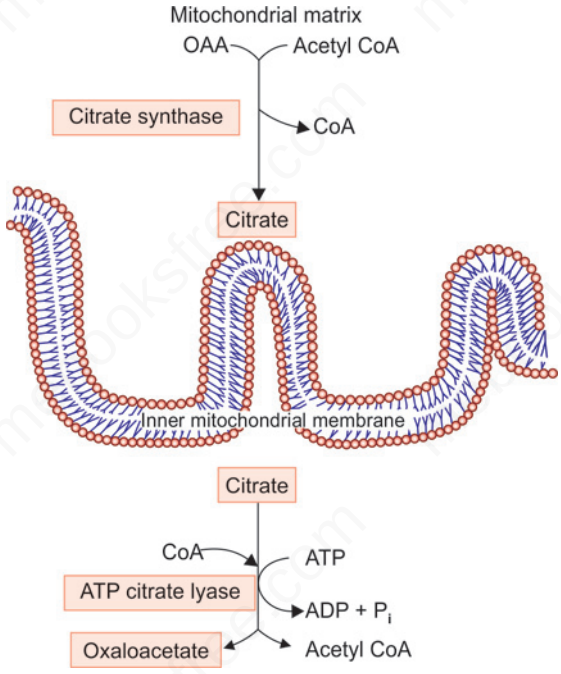


Fig. 7.6: Transport of acetyl CoA

- So Acetyl CoA is translocated by a shuttle mechanism.
- Acetyl CoA condenses with Oxaloacetate within the mitochondria to form Citrate.
- Citrate is translocated into cytosol via the **Tricarboxylate Transporter**.
- Citrate undergoes cleavage to acetyl-CoA and oxaloacetate catalyzed by **ATP Citrate Lyase.**

Fatty Acid Synthesis

By two enzyme system

1. Acetyl CoA Carboxylase
2. Fatty Acid Synthase Complex

Acetyl CoA Carboxylase

- Converts Acetyl CoA (2C) to Malonyl CoA in the presence of ATP.
- Acetyl-CoA carboxylase has a requirement for the B complex vitamin Biotin.
- It is a multienzyme protein.
- This is the rate limiting step[Q] in the Fatty Acid Synthesis.
- Acetyl CoA carboxylase is not a part FAS Complex.[Q]

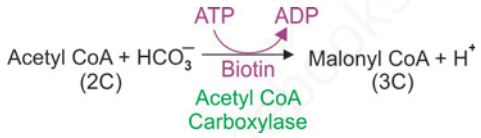


Fig. 7.7: Acetyl CoA carboxylase

Fatty Acid Synthase (FAS) Multienzyme Complex[Q]

- The complex is a homodimer of two identical polypeptide monomers in which six enzyme activities and the acyl carrier protein (ACP)
- ACP[Q] contains the vitamin **Pantothenic acid** in the form of 4′-phosphopantotheine
- X-ray crystallography of the three-dimensional structure, shown that the complex is arranged in an **X shape**.

Six enzyme activities of Fatty Acid Synthase Complex are

1. Ketoacyl Synthase
2. Malonyl-Acetyltransacylase
3. Hydratase
4. Enoyl Reductase
5. Ketoacyl Reductase
6. Thioesterase (Deacylase)

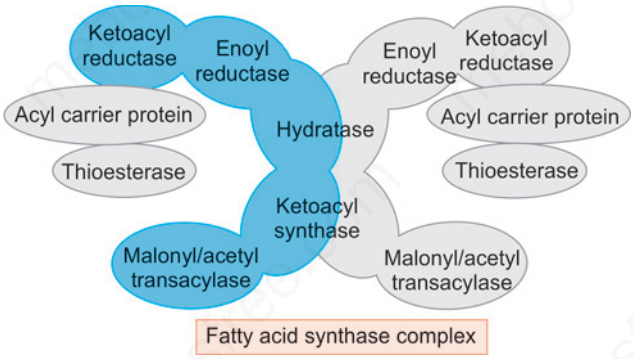


Fig. 7.8: Fatty acid synthase complex

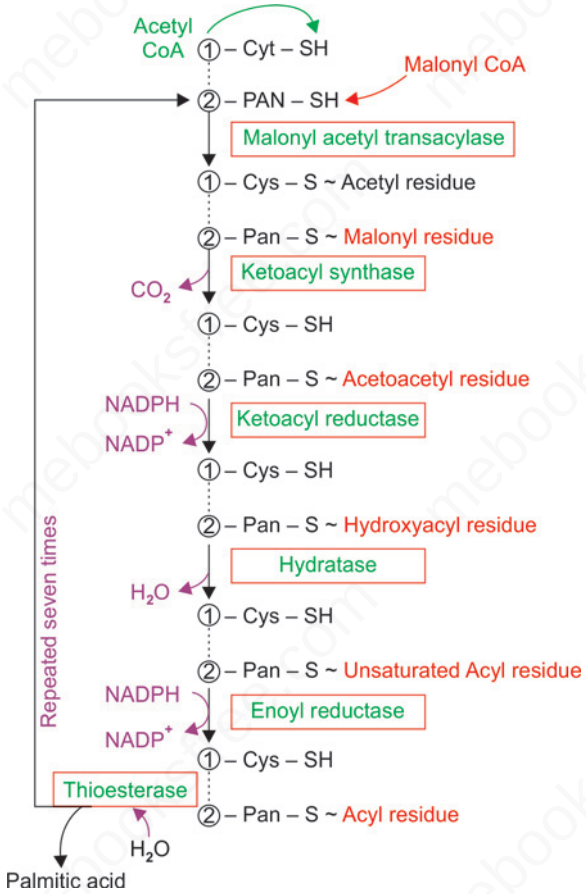


Fig. 7.9: De novo fatty acid synthesis

Sources of NADPH

- The main Source of NADPH for lipogenesis is the HMP Pathway (Pentose Phosphate Pathway)
- **Malic enzyme** (NADP malate dehydrogenase)
- The extra mitochondrial isocitrate dehydrogenase reaction.

Reactions of Fatty Acid Synthase Complex

By three stages

1. Condensation
2. Reduction
3. Release of Fatty acid

Condensation Reactions

- *Malony/Acetyl Transacylase:* A priming molecule of acetyl-CoA combines with a cysteine—SH group.
  - While malonyl-CoA combines with the adjacent SH on the 4′-phosphopantetheine of ACP of the other monomer.
  - These reactions are catalyzed by **malonyl** acetyl **transacylase**, to form **acetyl (acyl)-malonyl enzyme**.
- *Ketoacyl Synthase:* The acetyl group attacks the methylene group of the malonyl residue, catalyzed by 3-ketoacyl synthase, and **liberates CO_2**, forming 3-ketoacyl enzyme (acetoacetyl enzyme) (reaction 2), freeing the cysteine—SH group.

Reduction reactions

- *Ketoacyl Reductase:* The 3-ketoacyl group is reduced to Hydroxyacyl group by Ketoacyl Reductase
- *Hydratase:* Hydroxyacyl group is dehydrated to Unsaturated Acyl (Enoyl) group by Hydratase
- *Enoyl Reductase:* Unsaturated Acyl (Enoyl) group is reduced to Acyl group by Enoyl Reductase.

Releasing of fatty acid

These reactions of Condensation and reduction repeated several times till the desired acyl group is assembled on the enzyme

Thioesterase

- Fatty acid (Acyl group) is liberated from the enzyme complex by the activity of the sixth enzyme in the complex, thioesterase (deacylase)

Regulation of Fatty Acid Synthesis

Rate limiting Step: Acetyl CoA Carboxylase

Long Term Regulation

Control of Enzyme Synthesis by regulation of gene expression.

Short Term Regulation

- Allosteric regulation
- Covalent Modification

Allosteric regulation

- Positive Allosteric regulation or Allosteric Activation of Acetyl CoA Carboxylase by Citrate♀
- Citrate promotes the conversion of Acetyl CoA Carboxylase from an Inactive dimer to active polymeric form.

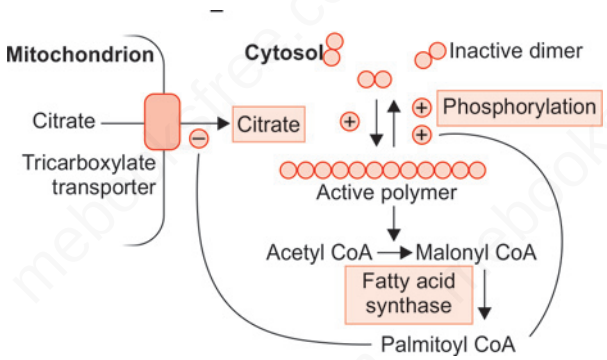


Fig. 7.10: Allosteric regulation of acetyl CoA carboxylase

Inactivation promoted by long chain Acyl CoA by:

- Favoring phosphorylation of Acetyl CoA Carboxylase
- By inhibiting the Tricarboxylic transporter that transport Citrate from mitochondria to Cytosol.

Covalent modification

- By phosphorylation-dephosphorylation by hormones
- Acetyl CoA Carboxylase is active in dephosphorylated state♀ and inactive in phosphorylated state.

Glucagon and epinephrine

Glucagon and Epinephrine inactivate Acetyl CoA Carboxylase by phosphorylating the enzyme.

Mechanism of Action of Glucagon and Epinephrine

- Glucagon/Epinephrine increase cAMP.
- cAMP favor cAMP dependent Protein Kinase.
- This activate AMP Kinase Kinase (AMP KK)
- AMPKK activate AMP Kinase (AMPK) by Phosphorylation.
- AMPK phosphorylate Acetyl CoA Carboxylase.

Insulin

Insulin activate Acetyl CoA Carboxylase, by dephosphorylating the enzyme.

Mechanism of Action of Insulin

- Insulin dephosphorylate AMP Kinase by dephosphorylation.
- This inturn dephosphorylate Acetyl CoA carboxylase.

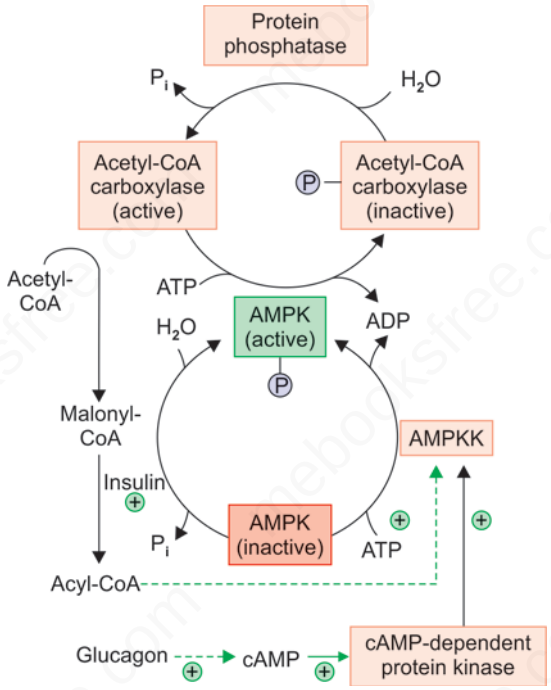


Fig. 7.11: Covalent modification of acetyl CoA carboxylase

Activation of acetyl CoA carboxylase	Inactivation of acetyl CoA carboxylase
Citrate	Acyl CoA
Insulin	Glucagon, Epinephrine
Dephosphorylation	Phosphorylation

Fates of Acyl CoA

Esterified into Triacyl Glycerol

- Chain Elongation to produce very long chain fatty acid
- Desaturation to produce unsaturated Fatty acid
- Esterified into Cholesterol Ester.

Elongation of Fatty Acid Chains

- Occurs in the Endoplasmic Reticulum (the 'microsomal system') and some in mitochondria also.
- By Fatty Acid Elongase system
- Elongates saturated and unsaturated fatty acyl-CoAs (from C10 upward) by two carbons
- Malonyl CoA donates 2 Carbon atoms in stepwise manner
- In the same manner as Fatty acid Synthase Complex in the Cytosol
- NADPH is required at the two reductase step
- Elongation reaction are particularly increased in brain during myelination to provide C22 and C24 fatty acids for sphingolipids.

Synthesis of Unsaturated Fatty Acids

- Occurs in Endoplasmic reticulum.
- By an enzyme called Fatty Acyl CoA Desaturase.
- The most common desaturase is Δ^9 Desaturase, a monoxygenase.
- The first double bond introduced is always in the Δ^9 position.
- Some unsaturated fatty acids are essential fatty acids.

Humans cannot introduce additional double bond in the fatty acid chain beyond Δ^9 (i.e. between C-10 and terminal methyl group. Hence, Linoleic Acid ($\Delta^{9,12}$) and Linolenic Acid ($\Delta^{9,12,15}$) become essential Fatty Acid.

Oxidation of Fatty Acid

The process by which fatty acids are successively cleaved to two carbon compound, Acetyl CoA and release energy.

***Different types of Fatty Acid Oxidation are*:**

- β Oxidation of Fatty Acid.
- Oxidation of Very Long chain Fatty Acids.
- Oxidation of Unsaturated Fatty Acids.
- Oxidation of Odd chain Fatty acid.
- α Oxidation of Fatty Acid.
- ω Oxidation of Fatty acid.

β Oxidation of Fatty Acid

- Most common type of fatty acid oxidation.ϙ
- Two carbon at a time are cleaved from Carboxyl end of activated Fatty acid as Acetyl CoA.ϙ
- The cut is between the α and β carbon atom and hence the name Beta oxidation.
- Site-Organs-Liver, Adipose Tissue, Muscle
- Organelle-Mitochondria

Steps of Fatty Acid Oxidation

- Activation of fatty Acids.
- Transport of activated fatty acid from Cytosol to mitochondria.
- Reactions of beta oxidation.

Activation of fatty acids

- **Site:** Cytoplasm
- Enzyme-Acyl CoA Synthetase/Thiokinase
- The only step in the complete degradation of fatty acid that require energy.
- Two inorganic phosphatesϙ are used.

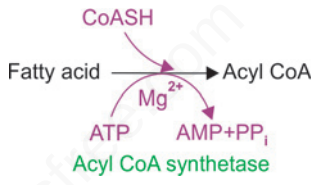


Fig. 7.12: Activation of fatty acid

Transport of Acyl CoA into Mitochondria

- Long-Chain Fatty Acids penetrate the Inner Mitochondrial Membrane as Carnitine^{Q} derivatives.
- Short Chain and Medium chain Fatty Acid does not need Carnitine for transport.

Carnitine

- Beta Hydroxy Gamma Trimethyl Ammonium Butyrate.
- Widely distributed particularly in the muscle.
- Synthesized from Lysine and Methionine in the liver.

Steps of transport of activated fatty acid

- Carnitine Acyltransferase I
  - Also called as Carnitine Palmitoyl Transferase-I [CPT-I) as most common fatty acid translocated is Palmitic acid.
  - Located in the Outer mitochondrial membrane.
  - Transfer acyl group present in the Acyl CoA to Carnitine to form Acyl Carnitine.
- Carnitine Acyl Carnitine Translocase
  - Acyl Carnitine is translocated across the inner mitochondrial membrane.
- Carnitine Acyltransferase II
  - Also called Carnitine Palmitoyl Transferase-II (CPT-II)
  - Located in the Inner mitochondrial membrane
  - Converts Acyl Carnitine to Acyl CoA.

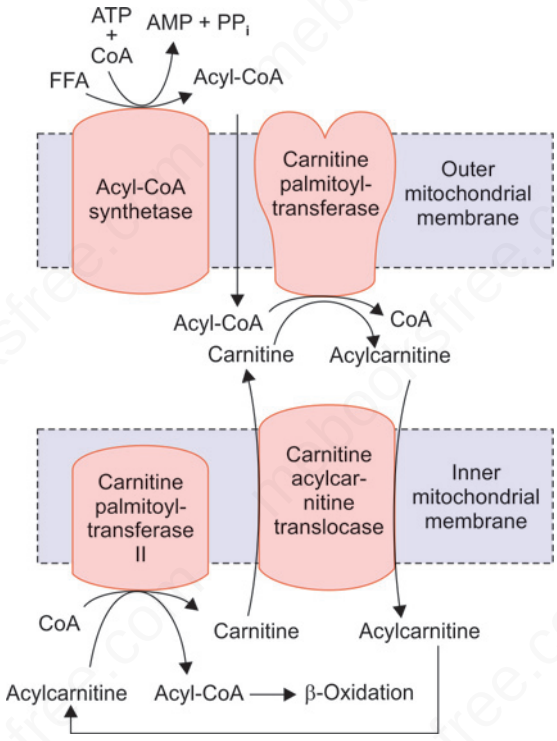


Fig. 7.13: Transport of fatty acid

- Carnitine Acyl Carnitine Translocase
  - Returns the Carnitine back to Cytosol.

Reactions of betaoxidation

Involves sequence of four reaction successively cleave 2 Carbons as **Acetyl CoA**

See Fig. 7.14 for reactions of Beta-oxidation.

Reaction	Enzyme	Reducing equivalents produced
Oxidation	Acyl CoA Dehydro-genase	FADH2 = 1.5 ATP
Hydration	Enoyl CoA Hydratase	—
Oxidation	Hydroxy Acyl CoA De-hydrogenase	NADH = 2.5 ATP
Cleavage	Thiolase	—

Energetics of Beta-Oxidation

The number of ATPs obtained depends on the number of Carbon atoms in the fatty acid.

From Beta-oxidation of Palmitic acid (C-16)

First calculate how many cycles of beta-oxidation [(n/2)-1] where n = no of carbon atoms

In case of palmitic acid, 7 cycles of beta oxidation

From one cycle of beta-oxidation

- 1 FADH2=1.5 ATPs
- 1 NADH=2.5 ATPs
- Total ATPs from 1 cycle of beta-oxidation = 4 ATPs

From 7 cycles of beta-oxidation

- 7 × No of ATPs from 1 cycle
- 7 × 4 = **28 ATPs**

Second calculate how many 2 Carbon Acetyl CoA from 16 Carbon Palmitic Acid

- (n/2) where n = no of carbon atoms

So in case of Palmitic Acid

- (16/2) = 8 Acetyl CoA
- From one Acetyl CoA by TCA Cycle 10 ATPs
- From 8 Acetyl CoA
- 8 × 10 = **80 ATPs**

Total ATPs from Palmitic Acid = 28 + 80 = 108

2 ATPs utilized for initial activation of Fatty Acid. **So net ATPs from Palmitic Acid = 108 – 2 = 106 ATPs**

From Stearic Acid (18C)

- 8 Cycles of Beta-oxidation + 9 Acetyl CoA
- (8 × 4) + (9 × 10) ATPs
- 32 + 90 = 122 ATPs

Net ATPs produced from stearic acid is 122 – 2 = 120 ATPs

Regulation of Beta Oxidation

Controlled by CPT-I Gateway

In the Fed State

- Increased Insulin/Glucagon ratio.
- Increased Acetyl CoA Carboxylase, Malonyl CoA is produced.
- Malonyl CoA is an inhibitor of CPT-I.
- So decreased Beta Oxidation.

In the fasting state

- Decreased Insulin/Glucagon ratio
- Decreased Acetyl CoA Carboxylase activity, Malonyl CoA is not increased
- Active CPT-I
- So increased Beta oxidation.

Concept—Regulation of Beta oxidation of Fatty acid

- When fatty acid synthesis takes place it inhibits its own oxidation, so that futile cycles will not operate.
- In fasting state, ATP can be provided by fatty acid oxidation as it is active.
- In fed state, body can store fat as fatty acid synthesis active.

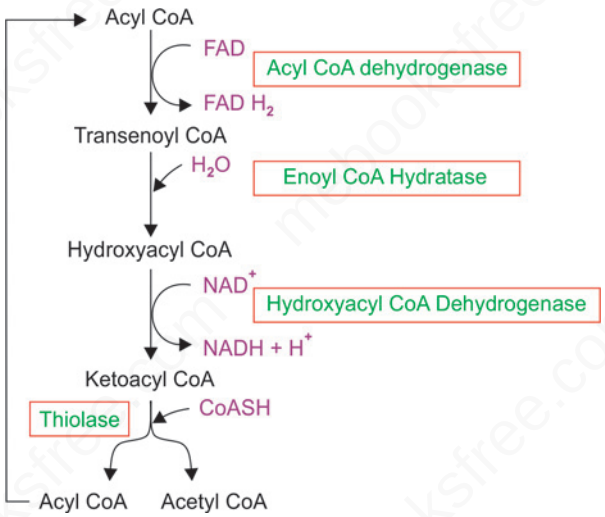


Fig. 7.14: Steps of beta oxidation

Clinical Correlations

Defects in β oxidation of fatty acids

Medium Chain Acyl CoA Dehydrogenase Deficiency (MCAD Deficiency)

- **Fasting Hypoglycemia**
- No Ketone Bodies
- Vomiting, Coma and Death
- C8-C10 Acyl Carnitine in the blood
- Episodes may be provoked by overnight fast in an Infant
- Primary Treatment is IV Glucose
- Prevention is by frequent feeding with high carbohydrate and low fat diet.

Fasting Hypoglycemia in MCAD deficiency

- On fasting ATP for Gluconeogenesis is provided by beta oxidation of Fatty acids.
- If MCAD deficiency fatty acid oxidation do not takes place and no ATP to support Gluconeogenesis. Hence, fasting hypoglycemia.

Sudden Infant Death Syndrome (SIDS) common in MCAD Deficiency

- Infants with MCAD deficiency if not fed for 12 hours or more especially in the nights, hypoglycemia sets in and death results.

Jamaican Vomiting Sickness

- Ackee fruit that grows in Jamaica and West Africa contain a toxin called Hypoglycin.
- Hypoglycin is an inhibitor of Fatty Acyl CoA dehydrogenase.
- Severe Hypoglycemia if ingested as it inhibit beta oxidation of fatty acids.
- Characterized by sudden onset of vomiting 2–6 hrs after ingestion, followed by convulsion, coma and death.

Carnitine Deficiency

Can occur particularly in the newborn—and especially in preterm infants and in hemodialysis.

Clinical features

- Hypoglycemia, which is a consequence of impaired fatty acid oxidation.
- Muscular weakness, due to lipid accumulation
- Hence they have a vitamin-like dietary requirement for carnitine .
- Treatment is by oral supplementation with carnitine

CPT-I Deficiency

- Affects only the liver, resulting in reduced fatty acid oxidation and ketogenesis, with hypoglycemia.

CPT-II Deficiency

- Affects primarily skeletal muscle and, when severe, the liver.

Sulfonyl Urea in Type II Diabetes Mellitus

- The sulfonylurea drugs **(glyburide [glibenclamide]** and **tolbutamide)**, used in the treatment of type 2

diabetes mellitus, reduce fatty acid oxidation by inhibiting CPT-I.
- Hence it reduces Gluconeogenesis, there by preventing Hyperglycemia.

Acute fatty liver of pregnancy
- Defects in long chain 3-hydroxyacyl-CoA dehydrogenase.

Oxidation of Very Long Chain Fatty Acid
- By a modified β oxidation pathway
- For Fatty Acids > C_{20}, C_{22}
- Takes place in the **Peroxisomes** till Octanoyl CoA
- Oxidation in peroxisome produces Acetyl CoA and H_2O_2 (instead of $FADH_2$)
- No ATP is generated
- Further oxidation of Acetyl CoA and Octanoyl CoA takes place in the **mitochondria.**
- Peroxisomes do not attack shorter chain fatty acids beyond Octanoyl CoA.
- Peroxisomal Oxidation shorten the side chains of Cholesterol in bile acid formation.
- Peroxisome take part in the synthesis of Cholesterol, and Dolichol and Ether Glycerolipids.

Clinical Correlation

Defects in the oxidation of VLCFA in the Peroxisomes

Peroxisomal disorders

The peroxisomal diseases are genetically determined disorders caused either by the failure to form or maintain the peroxisome or by a defect in the function of a single enzyme that is normally located in this organelle.

The basic defect is
- The proteins that are destined to the peroxisomes has a specific targeting sequence called peroxisome targeting sequence (PTS).
- Defects in the PTS leads to Peroxisomal Disorders.

Peroxisomal ghost
- Absence or reduction in the number of peroxisome is pathognomonic for disorders of peroxisome biogenesis.
- In most disorders there are membranous sacs that contain peroxisomal integral membrane proteins, which lack the normal complement of matrix proteins; these are Peroxisome 'ghosts'.

Some peroxisomal disorders are:
- Zellweger's syndrome
- Neonatal adrenoleukodystrophy (NALD)
- Infantile Refsum disease (IRD)
- Rhizomelic chondrodysplasia punctata (RCDP)

Lorenzo's oil therapy
- Treatment for Adrenoleuko Dystrophy
- Lorenzo's oil (4:1 mixture of glyceryltrioleate and glyceryltrierucate)

Oxidation of Unsaturated Fatty Acids
- Occurs by a modified β-oxidation Pathway.
- In the mitochondria.
- Till the double is reached, normal beta oxidation will take place.
- An additional Isomerase and a Reductase helps to shifts the double bond.
- The first step, FAD dependent Acyl CoA dehydrogenase is bypassed.
- $FADH_2$ is not formed.
- The energy yield by oxidation of Unsaturated Fatty Acid is **1.5 ATP less per double bond**.

Oxidation of Odd Chain Fatty Acid
- Takes place in the mitochondria.
- Oxidation of a Fatty Acid with an odd number of carbon atoms yields Acetyl CoA and a molecule of **Propionyl-CoA**
- The propionyl residue from an odd-chain fatty acid is the only part of a fatty acid that is glucogenic.

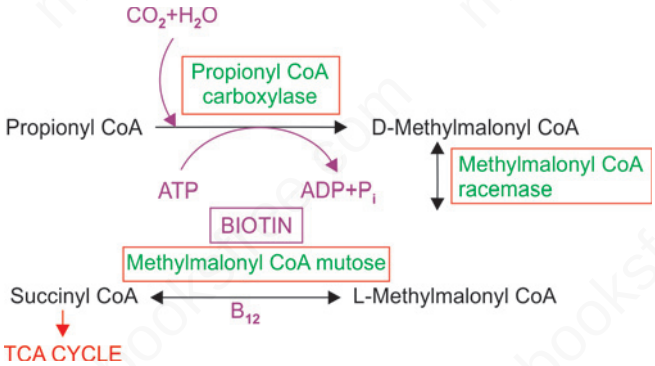


Fig. 7.15: Conversion of propionyl CoA to succinyl CoA

Minor Oxidation Pathways of Fatty Acid

α-Oxidation of fatty acids
- Site: Endoplasmic reticulum and Peroxisome.
- Removal of one carbon at a time from the α Carbon atom.
- For oxidation of Branched chain Fatty Acid to remove methyl group at the branch points.
- Neither consumes nor generate energy.

- Fatty Acid does not need activation.
- Used for oxidation of Phytanic acid, a major dietary methylated fatty acid seen in dairy products.

Refsum's disease

- Defect in Alpha oxidation of Phytanic Acid (Phytanic Acid Oxidase) (phytanoyl CoA hydroxylase) in the Peroxisome.
- The manifestation of classic Refsum's Disease includes impaired vision from retinitis pigmentosa, ichthyosis, peripheral neuropathy, ataxia, and, occasionally, cardiac arrhythmias.
- Classic Refsum disease often does not manifest until young adulthood, but visual disturbances such as night blindness, ichthyosis, and peripheral neuropathy may already be present in childhood and adolescence.
- Restrict dietary dairy products and Green Leafy Vegetables.

ω-Oxidation of fatty acid

- Occur in the Endoplasmic Reticulum.
- Oxidation involves methyl group at the ω end.
- Involves Hydroxylation at the terminal methyl group at the ω end by CytP450 (Mixed function Oxidase).
- Resulting in Short chain Dicarboxylic Acid (double headed fatty acid)

Quick Review—Sites of Oxidation of Fatty acids	
Beta oxidation of fatty acid	Mitochondria
Beta oxidation of unsaturated fatty acids	Mitochondria
Beta oxidation of very long chain fatty acid	Peroxisomes up to Octanoyl CoA, then rest in mitochondria
Alpha oxidation of fatty acid	Peroxisomes, Smooth Endoplasmic reticulum
Omega oxidation of fatty acid	Smooth Endoplasmic Reticulum
Activation of fatty acid	Cytosol

Ketone Bodies

Ketogenesis occur in metabolic conditions associated with high rate of fatty acid oxidation.

- Primary Ketone Body is Acetoacetate
- Secondary Ketone bodies are Acetone and Beta Hydroxy Butyrate.
- Concentration of Ketone Bodies in the blood does not normally exceed 0.2 mmol/L.
- In normal persons the ratio of beta hydroxy butyrate to acetoacetate is 1:1
- In Ketosis the ratio of beta hydroxy butyrate to acetoacetate is 6:1.

Ketone Body Synthesis

Site: Exclusively Liver Mitochondria[Q]

- **Acetoacetyl CoA** from Beta oxidation is the starting material.
- **HMG CoA Synthase** is the rate limiting step.
- HMG CoA Synthase is common to Cholesterol Synthesis and Ketone Body Synthesis.

Steps of Ketone Body Synthesis

- Two acetyl-CoA molecules formed in β-oxidation condense to form acetoacetyl-CoA by a reversal of the **thiolase** reaction.
- Condensation of acetoacetyl-CoA with another molecule of acetyl-CoA by **Hydroxy-3-methylglutaryl-CoA synthase** forms 3-hydroxy-3-methylglutaryl-CoA **(HMG-CoA).**
- **Hydroxy-3-methylglutaryl-CoA lyase** then causes acetyl-CoA to split off from the HMG-CoA, leaving free acetoacetate.
- Acetoacetate continually undergoes spontaneous decarboxylation to yield acetone.
- Acetoacetate converted to β Hydroxybutyrate by the mitochondrial enzyme β **hydroxybutyrate dehydrogenase**.

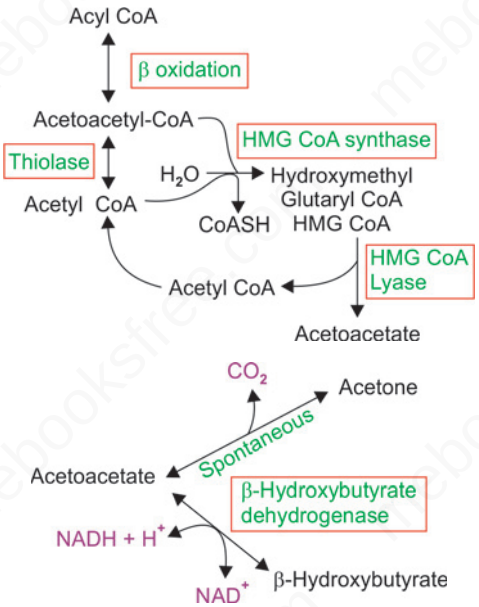


Fig. 7.16: Ketone body synthesis

The Pathways where HMG CoA is an Intermediate are:

- Ketone Body Synthesis
- Cholesterol Synthesis
- Leucine Catabolism.

Energetics of Fatty acid oxidation if Ketone bodies are the end products
- 26 mol of ATP are produced when acetoacetate is the end product
- 21 mol when β-hydroxybutyrate is the end product.

Utilization of Ketone Bodies

Ketone Bodies serve as a fuel for extrahepatic tissues.

Almost all the organs utilize Ketone bodies with the exception of **Liver and RBCs.**Q

Liver does not utilize Ketone Bodies.

Steps of utilization of Ketone Bodies
- In extrahepatic tissues, acetoacetate is activated to **acetoacetyl-CoA by succinyl-CoA-acetoacetate CoA transferase or Thiophorase.**
- CoA is transferred from succinyl-CoA to form acetoacetyl-CoA
- The acetoacetyl-CoA is split into two acetyl-CoAs by thiolase
- Acetyl CoA is oxidized in the citric acid cycle.
- Acetone is difficult to oxidize in vivo and to a large extent is volatilized in the lungs.

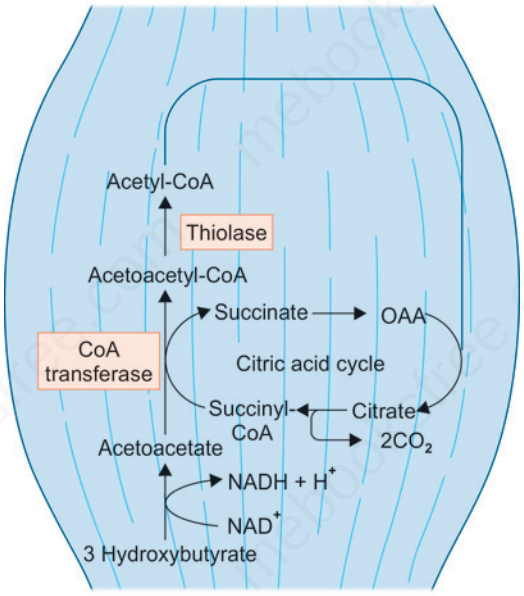


Fig. 7.17: Utilization of ketone bodies

Test for Ketone Bodies in urine
- Gerhardt's Ferric Chloride Test-Answered by Acetoacetate
- Rothera's Nitroprusside Test-Answered by Acetoacetate and Acetone
- NB:-None of these tests answer Betahydroxybutyrate the predominant ketone body in Ketosis.

Quick Review—Ketone bodies
- Primary Ketone body-Acetoacetate
- Secondary Ketone bodies-Acetone, Betahydroxybutyrate
- Neutral Ketone body-Acetone
- Ketone body excreted through lungs-Acetone
- Site of synthesis of Ketone bodies-Liver mitochondria
- Organs which do not utilize ketone bodies-Liver, RBCs
- Rate limiting step of Ketone body synthesis-HMG CoA Synthase
- Ketone bodies that do not answer Gerhadts test-Betahydroxybutyrate and Acetone.
- Ketone body that does not answer neither Rothera test nor Gerhadts test-Betahydroxybutyrate

Fuels for Different Organs in Fed and Fasting State

Organ	Fed	Fasting	Starvation
Brain	Glucose	Glucose	Ketone Bodies
Heart	Fatty Acid	Fatty Acid	Ketone Bodies
Liver	Glucose	Fatty Acid	Amino Acid
Muscle	Glucose	Fatty Acid	Fatty Acid/Ketone Bodies
RBC	Glucose	Glucose	Glucose

CLINICAL CORRELATION: FATTY LIVERQQQ

Lipid mainly as **triacylglycerol**Q can accumulate in the liver, called fatty liver.

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disorder worldwide.

Stages of progression of NAFLD

Nonalcoholic steatohepatitis (NASH), *which can progress to liver diseases including* cirrhosis, hepatocarcinoma, and liver failure.

Causes of Fatty LiverQ
- The basic cause of fatty liver is imbalance between rate of Triacyl Glycerol synthesis and its export in the liver.

Fatty Liver fall into two main categories

1. *Due to raised levels of plasma free fatty acids:* The production of VLDL does not keep pace with the increasing influx and esterification of free fatty acids, allowing triacylglycerol to accumulate, which in turn causes a fatty liver.

This can be due to
- Starvation
- The feeding of high-fat diets
- In uncontrolled diabetes mellitus
- Twin lamb disease
- Ketosis in cattle.

2. *Due to a metabolic block in the production of plasma lipoproteins, thus allowing triacylglycerol to accumulate.*

This may be due to:

- A block in apolipoprotein synthesis as in Kwashiorkar
- A block in the synthesis of the lipoprotein from lipid and apolipoprotein
- A failure in provision of phospholipids that are found in lipoproteins
- A failure in the secretory mechanism itself
- **Orotic acid** also causes fatty liver
- The antibiotic puromycin, ethionine, carbon tetrachloride, chloroform, phosphorus, lead, and arsenic all cause fatty liver
- **Lack of Lipotropic Factors**[Q]

Lipotropic Factors are:

- Choline, Betaine
- **Vitamin E-**supplemented diets.
- Methionine in S Adenosyl Methionine,trap the available adenine and prevent the synthesis of ATP.
- **Selenium**
- Essential fatty acid, e.g. linoleic acid
- Pyridoxine, and Pantothenic acid

Alcoholic Fatty Liver

Alcoholic fatty liver is the first stage in **alcoholic liver disease (ALD)** which is caused by **alcoholism** and ultimately leads to **cirrhosis.**

The fat accumulation in the liver is caused by a combination of impaired fatty acid oxidation and increased lipogenesis.

Oxidation of ethanol by **alcohol dehydrogenase, Aldehyde Dehydrogenase** leads to excess production of NADH.

This results in increased NADH/NAD^+ ratio. This results in

- Increased esterification of fatty acids to form triacylglycerol, resulting in the fatty liver.
- Increased (lactate)/(pyruvate), resulting in **hyperlacticacidemia,** which decreases excretion of uric acid, aggravating **gout.**

CHOLESTEROL

- Exclusive animal sterol never seen in plants
- Major component of Plasma membrane
- Made up of Steroid nucleus called 'Cyclopentanoperhydrophenanthrene (CPP)'
- Total number of carbon atom in Cholesterol is 27
- Amphipathic.

Cholesterol Synthesis

Major Sites: All tissues containing nucleated cells are capable of Cholesterol Synthesis especially in Liver, Adrenalcortex, Testes, Ovaries, Intestine

Orgenelle: Smooth Endoplasmic Reticulum and Cytoplasm

Starting Material: Acetyl CoA.

Steps of Cholesterol Synthesis

Formation of HMG CoA

- Two molecules of Acetyl CoA (2C) condense to form Acetoacetyl CoA (4C) by the enzyme Thiolase.
- Acetoacetyl CoA condense with a third molecule of Acetyl CoA to form HMG CoA by the enzyme HMG CoA Synthase.

Synthesis of Mevalonate

- HMG CoA (6C) converted to Mevalonate (6C) by HMG CoA Reductase
- ***This is the rate limiting step***[QQQ]
- Takes place in the Endoplasmic reticulum.
- NADPH is required.
- Statins are competitive inhibitor of HMG CoA Reductase.

Generation of Isoprenoid Units (5C)

- Mevalonate on decarboxylation and phosphorylation to form Isoprenoid units.

Condensation of 5 Carbon isoprenoid units to form Squalene (30C)

- Two 5C unit condense to form 10C compound– Geranyl Pyrophosphate.
- 10C unit, Geranyl Pyrophosphate condense with a 5C Isoprenoid unit to form 15C compound-Farnesyl Diphosphate.
- Two Farnesyl Diphosphate (15C) condense to form 30C compound-Squalene.

Formation of Cholesterol

- Linear 30C molecule cyclises to form a structure that closely resembles steroid nucleus called Lanosterol.
- Lanosterol undergo further modification to form Cholesterol (27C)
- The intermediates in the conversion of Lanosterol to Cholesterol are
  - 14 Desmethyl Lanosterol
  - Zymosterol
  - Desmosterol

Lanosterol

- First Cyclical Compound formed
- First Steroid Compound formed

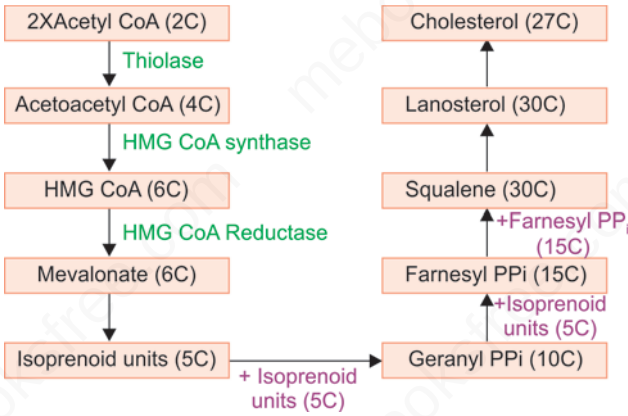


Fig. 7.18: Synthesis of cholesterol

Compare–Cholesterol Synthesis and Ketone Body Synthesis

Characteristics	Ketone body synthesis	Cholesterol synthesis
Site	Mitochondria	Cytoplasm/Smooth Endoplasmic reticulum
HMG CoA as an intermediate	Yes	Yes
HMG CoA Synthase	Yes, the regulatory step	Yes
HMG CoA Reductase	No	Yes, the rate limiting step
HMG CoA Lyase	Yes	No

Remember
- Cytoplasmic HMG CoA Synthase is for Cholesterol synthesis
- Mitochondrial HMG CoA Synthase is for Ketone body synthesis

Uses of Isoprenoid units in Farnesyl and Geranyl Diphosphate

- Polyisoprenoid compounds **Dolichol and Ubiquinone** are formed from Farnesyl Diphosphate.
- **Prenylation** of Proteins:
  - It is a Post Translational Modification.
  - GTP Binding Proteins are prenylated.
  - Facilitate anchoring of proteins to lipoid membranes.
  - Facilitate Protein Trafficking.

Regulation of Cholesterol Synthesis

Rate limiting Enzyme is HMG CoA Reductase.

- *Feed back inhibition:* HMG CoA Reductase is inhibited by Mevalonate and Cholesterol.
- *Feedback regulation:* Cholesterol repress the transcription of genes for HMG CoA Reductase.
  - This acts via Sterol Regulatory element binding protein (SREBP)
- *Hormonal Regulation:* By Covalent modification-Phosphorylation dephosphorylation
  - HMG CoA Reductase is active in dephosphorylated state and vice versa.
  - **Insulin and Thyroxine increase the activity of HMG CoA Reductase**
  - **Glucagon and Glucocorticoids decrease the activity of HMG CoA Reductase**

Mechanism of regulation by Insulin

Insulin has a dominant role in regulation than Glucagon. Favor HMG CoA Reductase by dephosphorylating the enzyme.

By two mechanisms

First method

- Insulin activates Protein Phosphatase.
- This converts active AMP Kinase to inactive AMP Kinase.
- So HMG CoA Reductase cannot be phosphorylated.
- Hence HMG CoA Reductase is in active dephosphorylated state.

Second Method

- Insulin converts HMG CoA Reductase directly to active dephosphorylated state.

Glucagon and Glucocorticoids

- Inhibit HMG CoA Reductase by phosphorylating the enzyme.
- By a cAMP mediated process, which inhibit Protein Phosphatase.
- So AMP Kinase is active which phosphorylate HMG CoA Reductase.
- HMG CoA Reductase is inactive.

Key points Cholesterol regulation
- Dietary cholesterol inhibit its own synthesis by repressing HMG CoA Reductase enzyme.
- Decrease of 100 mg of dietary cholesterol cause a decrease of approximately 0.13 mmol/L of serum cholesterol.
- HMG CoA reductase is active in dephosphorylated state.
- Insulin and Thyroxine favor Cholesterol synthesis.
- Glucagon and Glucocorticoids inhibit cholesterol synthesis.

Tests for Cholesterol

- Liebermann Burchard test.
- Salkowski's Test.
- Zlatki Zak's Reaction.

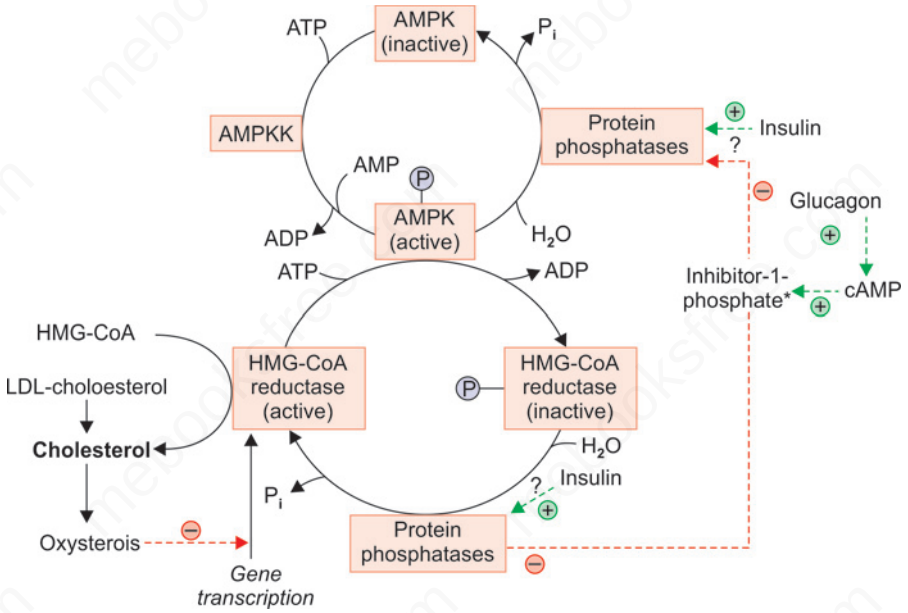


Fig. 7.19: Regulation of cholesterol synthesis

Cholesterol cannot Generate Energy

- Unlike other biomolecules, cholesterol does not degrade to generate energy.

The Fates of Cholesterol

- Nearly half converted to Bile acids.
- Some excreted in feces as cholestanol and coprostanol.
- Coprostanol is the principal sterol in the feces.
- Rest serve as precursors of Vitamin D and Sex hormones, Corticosteroids

Specialized Products of Cholesterol

- Bile Acids (Excretory form of Cholesterol)
- Vitamin D
- Sex Hormones
- Corticosteroids

BILE ACID SYNTHESIS

Starting material-*Cholesterol*ᑫ

Bile Acids and its Site of Synthesis

Primary Bile acids—Liver

They are:

- Cholic Acid(Most abundant bile acids in mammals)
- Chenodeoxycholic Acid or Chenic acid

Secondary Bile acids-Intestine

They are:

- Deoxycholic Acid
- Lithocholic Acid

Steps of Bile Acid Synthesis

- **Synthesis of Primary Bile Acids—In the Liver**

  First step:

  – Cholesterol converted to 7 Hydrocholesterol by 7 α Hydroxylase, a microsomal Cyt P450 enzyme designated as CYP7A1.

  – A typical monooxygenase requires oxygen, NADPH and Vitamin C.

  – This is the rate limiting step.ᑫ

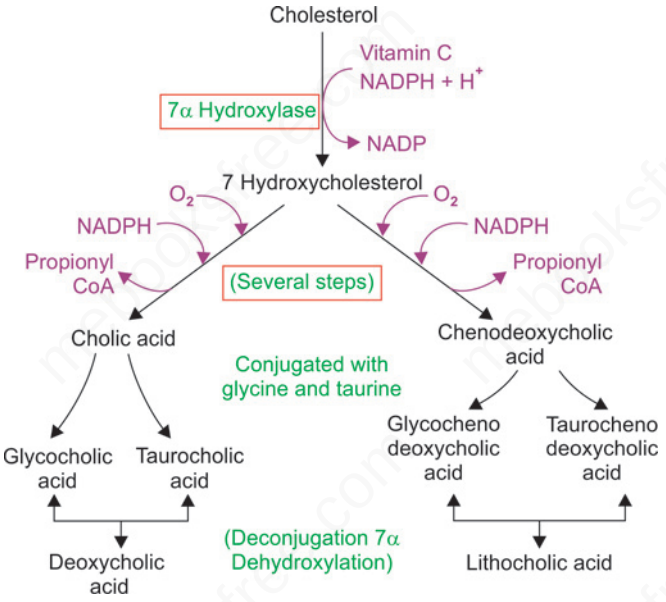


Fig. 7.20: Bile acid synthesis

Further multiple steps:
- 7 Dehydrocholesterol is divided into two subpathways leading to synthesis of Cholic acid and Chenodeoxy Cholic acid.

- Conjugation of Primary Bile Acids
  - Primary bile acids are conjugated with Glycine or Taurine.
  - Conjugation takes place in liver peroxisomes.
  - In humans the ratio of Glycine to Taurine conjugates is 3:1.

With glycine	With taurine
Glycocholic Acid	Taurocholic acid
Glycochenodeoxycholic acid	Taurochenodeoxycholic Acid.

Bile Salts
- In alkaline bile (pH 7.6 to 8.4) usually conjugated bile acids exists as sodium and Potassium salts
- So they are called bile salts.
- Bile salts enter into liver through bile.

- **Synthesis of Secondary Bile Acids—In the Intestine**
  Intestinal bacterial enzymes deconjugate and dehydroxylate primary bile acids to form secondary bile acids.
  - Cholic acid to Deoxycholic acid
  - Chenodeoxycholic acid to Lithocholic acid
- Enterohepatic Circulation
  - Primary and Secondary bile acids are absorbed exclusively in the ileum.
  - 98–99% of absorbed bile acids are returned to the liver via portal circulation (Enterohepatic Circulation).
  - Lithocholic acid is the bile acid which undergo least enterohepatic circulation.

Regulation of Bile Acid Synthesis
- The principal rate-limiting step in the biosynthesis of bile acids is at the cholesterol 7α- hydroxylase **(CYP7A1) reaction.**
- The activity of the enzyme is feedback regulated via the nuclear bile acid-binding receptor**, farnesoid X receptor (FXR).**
- When the size of the bile acid pool in the enterohepatic circulation increases, FXR is activated, and transcription of the cholesterol 7α-hydroxylase gene is suppressed.
- Chenodeoxycholic acid is particularly important in activating FXR.
- Cholesterol 7-hydroxylase activity is also enhanced by cholesterol of endogenous and dietary origin.

LIPOPROTEINS (VERY IMPORTANT)

Definition
- Lipoproteins are compound lipids formed as a combination of lipids with proteins.
- The protein part of lipoprotein is called apolipoprotein.
- Helps to transport lipids in the plasma.

Structure of lipoprotein
- Lipoproteins consist of a nonpolar core and a single surface layer of amphipathic lipids
- The nonpolar lipid core consists of mainly triacylglycerol[Q] and cholesteryl ester[Q]
- It is surrounded by a single surface layer of amphipathic phospholipid and cholesterol molecules.
- These are oriented so that their polar groups face outward to the aqueous medium, as in the cell membrane.

Major Classes of Lipoproteins
Based on ultracentrifugation, in the ascending order of density is
- Chylomicrons (Least Density)
- Very Low Density Lipoproteins (VLDL)
- Low Density Lipoproteins (LDL)
- Intermediate density lipoproteins (IDL)
- High Density Lipoproteins (HDL)

Based on electrophoretic separation

From cathode to anode the order of Lipoprotein in an electrophoretogram is (See Fig. 7.21)
- Chylomicron
- LDL (β Lipoprotein)
- VLDL (Pre β Lipoprotein)
- IDL (Broad β Lipoprotein)
- HDL (α Lipoprotein)

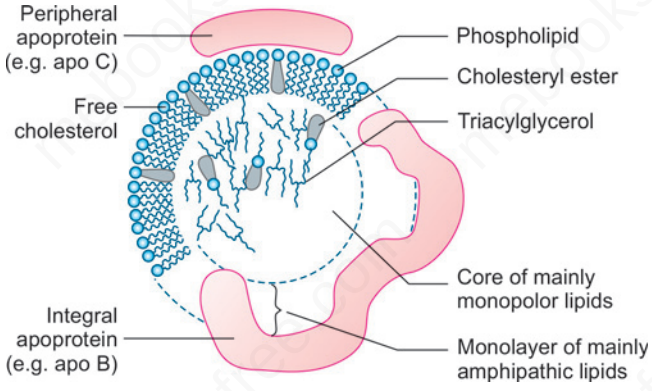


Fig. 7.21: Structure of lipoprotein

Separation of lipoproteins in an electric field depends on the *protein content*

- Higher the protein content faster the mobility of lipoprotein in the electric field.
- Chylomicron[9] with least protein content remains at the origin and HDL with highest protein content moves fastest.
- An exception is VLDL and IDL with less protein content moves ahead of LDL.

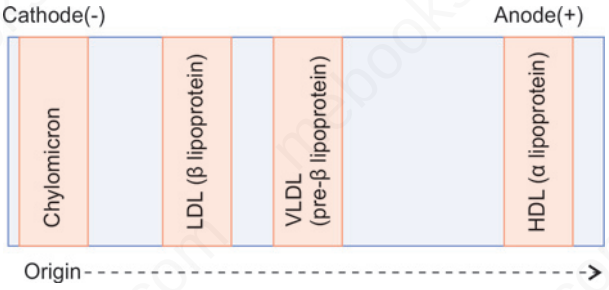


Fig. 7.22: Electrophoretic separation of lipoproteins

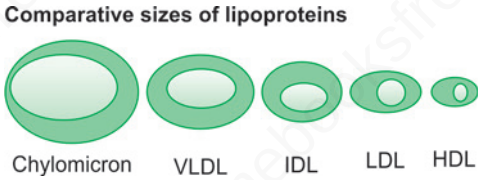


Fig. 7.23: Size of lipoproteins

Lipoproteins—Composition and Apolipoproteins

Lipoprotein	Apolipoproteins	Composition	
		Protein (%)	Lipid (%)
Chylomicron	apo B48 apo C-I,C-II,C-III apo E apo A-I,A-II,A-IV	1–2	98–99
VLDL	apo B100 apo C-I,C-II,C-III apo E	7–10	90–93
IDL (VLDL Remnant)	apo B100 apo E	11	89
LDL	apo B100	21	79
HDL	apo A-I,A-II,A-IV apo C-I,C-II,C-III apo D & E	32–57	43–68

Lipoproteins and its function

Lipoprotein	Function
Chylomicron	Formed in the Intestine. Carry dietary Triacylglycerol (Exogenous TAGs) to the liver.
VLDL	Formed in the liver. Carry endogenous Triacylglycerol
IDL (VLDL Remnant)	Formed from VLDL. LDL is formed from IDL.
LDL	Derived from VLDL remnant. Deliver Cholesterol and cholesterol ester to extrahepatic tissues and to liver.
HDL	Formed in the liver and intestine. Deliver cholesterol from peripheral tissues to liver and other steroidogenic tissues.[q]

Apolipoproteins

- The protein part of Lipoprotein is apolipoprotein abbreviated as apo.
- They are integral protein (e.g. apo B) which cannot be removed to other lipoprotein or peripheral proteins (e.g. apos C and E).
- The major apolipoprotein in HDL is apo A.
- The major apolipoprotein in LDL and VLDL is apo B100.
- Chylomicron contain a truncated apolipoprotein apo B48.

Apolipoproteins and its Function

Apolipoprotein	Function
Apo A-I	Activates Lecithin Cholesterol Acyl Transferase (LCAT)
Apo A-II	Inhibits Lipoprotein Lipase
Apo A-V	Promote lipoprotein lipase mediated Triacyl Glycerol lipolysis.
Apo B-100	Assembly of VLDL in the liver. Act as ligand for the LDL receptor and LDL receptor related protein (LRP-1) for uptake of LDL
Apo B-48	Assembly of Chylomicron in the intestine.
Apo C-I	Inhibit Cholesterol Ester Transfer Protein (CETP)
Apo C-II	Activates lipoprotein Lipase
Apo C-III	Inhibit Lipoprotein Lipase
Apo E	Act as ligand for LDL receptor for uptake of Chylomicron remnant and VLDL remnant (IDL)

More about Apolipoproteins

Apo E is rich in Arginine.

Apo D is associated with human neurodegenerative disease.

Isoforms of apo E

- Apo E gene is polymorphic in sequence.
- This result in expression of three common isoforms.
- Apo E2, Apo E-3, Apo E-4
- Apo-E3 is most common.
- Individuals carry two apo E-4 alleles are prone to develop Alzheimers disease.
- Apo E-2 has a low affinity for LDL-receptor.
- Individuals carrying two apo E-2 allele is prone to develop type III hyperlipoproteinemia (Familial Dysbeta Lipoproteinemia)

Lipoproteins at a Glance

Chylomicron

- Maximum Diameter.
- Least Density.
- Most buoyant lipoprotein.
- Least Protein content.
- Maximum Triacylglycerol.
- Least Electrophoretic Mobility [Remain at the point of application].
- Least plasma half life.
- Carry exogenous (dietary Triacylglycerol from Intestine to Peripheral tissues).
- Carry dietary cholesterol and cholesterol ester into the liver.

VLDL

- Prebeta lipoprotein
- Carry endogenous TAG from Liver to peripheral Tissues

IDL

- Otherwise called VLDL remnant
- Broad Beta Lipoprotein

LDL

- Beta Lipoprotein.
- Maximum Cholesterol and Cholesterol ester.
- 70% of LDL is degraded in liver and 30% of LDL degraded in extrahepatic tissue.
- LDL receptor is responsible for it.
- The degradation of LDL in extra hepatic tissue is responsible for deposition of cholesterol and cholesterol ester in the extrahepatic tissues.
- This makes LDL cholesterol 'the bad cholesterol.'

HDL

- Alpha lipoprotein
- Least Diameter
- Maximum Electrophoretic Mobility
- Maximum Protein Content
- Carry Cholesterol from peripheral tissues to liver and other steroidogenic tissues
- This is called Reverse Cholesterol transport
- This makes HDL Cholesterol 'the good cholesterol'
- The major role of HDL is to acts as the repository for apo C and apo E required for the metabolism of VLDL and Chylomicron.

Lp (a)

- Almost silmilar to LDL.
- Apo (a) is attached to apo B100 by disulphide bond.
- Major site of clearance of Lp(a) is liver.
- Strongly associated with Atherosclerosis and Myocardial infarction.
- Significant homology with Plasminogen[Q].
- It interferes with activation of Plasminogen to plasmin.
- Hence fibrin clot is not lysed.
- Susceptible to Intravascular thrombosis.

Lpx[NBE patternQ]

- Cholesterol is excreted as bile acids in the bile.
- In cholestasis, cholesterol combines with Phospholipid and form Lipoprotein X.
- Hence it is an index of cholestasis.

LIPOPROTEIN METABOLISM

Chylomicron Metabolism

Step I—Formation of Nascent Chylomicron

Assembly of nascent chylomicron in the intestine transported by lymphatics.

Step II—Formation of Mature Chylomicron

Remodelled to mature chylomicron by receiving apo C-II and apo E from HDL.

Remember

HDL acts as the repository of apo C and apo E.

Step III—Formation of Remnant Chylomicron

- Apo C-II activates Lipoprotein Lipase
- Lipoprotein lipase that is located on the walls of blood capillaries, anchored to the endothelium by negatively charged proteoglycan chains of Heparan Sulfate.

- Lipoprotein Lipase hydrolyses TAG in mature chylomicron to fatty acid and glycerol, to form remnant Chylomicron.
- Fatty acid delivered to these tissues for storage.
- Thus Chylomicron remnant is formed.

Step IV-Uptake of Remnant Chylomicron.

- Chylomicron remnant is taken up in the liver by receptor mediated endocytosis.
- Uptake is mediated by apo E via two apo E dependent recptors,LDL receptor and LDL receptor related protein-I (LRP-I)
- Hepatic Lipase hydrolyse remnant triacylglycerol and phospholipid.

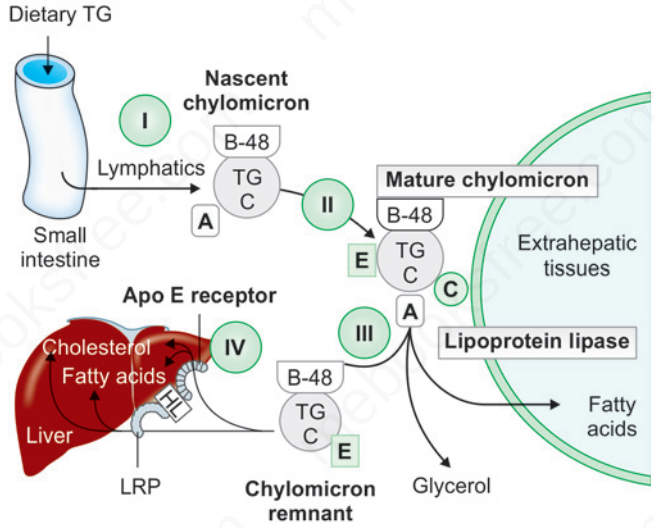


Fig. 7.24: Metabolism of chylomicron

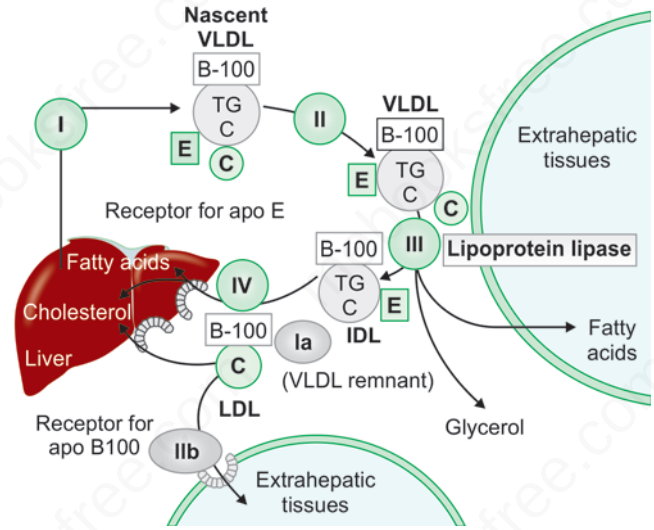


Fig. 7.25: Metabolism of VLDL

VLDL Metabolism

Step I—Formation of Nascent VLDL

- Assembly of nascent VLDL from the liver which carry endogenous Triacylglycerol.

Step II—Formation of Mature VLDL

- Nascent VLDL remodelled to mature VLDL by receiving apo C-II and apo E from HDL.

Step III—Formation of Remnant VLDL (IDL)

- apo C-II activates Lipoprotein Lipase
- Lipoprotein lipase that is present in the walls of the capillaries that lines the extrahepatic tissues hydrolyses TAG to Fatty Acid and Glycerol.
- Fatty acid delivered to these tissues for storage.
- Thus VLDL remnant (IDL) is formed.

Step IV—FATES OF IDL

- VLDL remnant (IDL) is taken up in the liver by apo E Receptor present in the liver.
- VLDL remnant is transformed to LDL particles. This is called Lipoprotein cascade pathway, i.e. VLDL to VLDL remnant (IDL) to LDL.



Fig. 7.26: Lipoprotein cascade pathway

Step V—Uptake of LDL by tissues

- LDL is metabolized by LDL receptor via receptor mediated endocytosis.
- apo B100 acts as the ligand for LDL receptors.
- Approximately 30% of LDL is degraded in extrahepatic tissues and 70% in the liver.

LDL Receptor

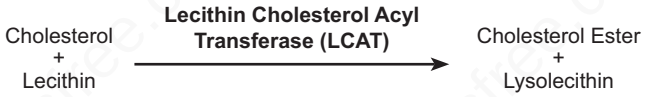
- LDL Receptor is a Glycoprotein.
- Ligand for LDL receptor are apo B100 and apo E.
- Present in **hepatic** and **extrahepatic tissues**.
- Occur on the cell surface in the pits coated on the cytosolic side of the cell membrane with a protein called **Clathrin.**
- LDL is taken intact by **Receptor mediated Endocytosis.**
- After uptake of LDL, the receptors are recycled to the cell surface.
- The influx of Cholesterol into the cell **suppress the synthesis of LDL receptor** by SREBP (sterol regulatory element-binding protein) pathway.
- Cholesterol lowering effect of **PUFA and MUFA** is thought to be due to **upregulation of LDL-receptor**, that increases the catabolic rate of cholesterol laden LDL.

HDL Metabolism and Reverse Cholesterol Transport

- Nascent HDL is synthesized and secreted from intestine and liver. Nascent HDL is discoidal in

shape, consist of phospholipid bilayer, Cholesterol and apo A.

- Lecithin Cholesterol Acyl Transferase (LCAT) binds to nascent HDL.
- Apo A-I activates LCAT.
- LCAT converts Cholesterol to nonpolar cholesterol ester.



- Cholesterol ester is nonpolar. So a nonpolar core is generated forming a spherical HDL (HDL_3)with surface film of amphipathic lipids and apolipoproteins.
- HDL_3 accepts Cholesterol from tissues by Class B Scavenger Receptor B-I (SR-BI) and ATP-binding cassette transporters A1 (ABCA1) and G-I (ABCG-I)
- LCAT acts on the Cholesterol in HDL3, convert it into cholesteryl esters.
- Thus less dense HDL_2 is formed.
- HDL_2 delivers Cholesterol and cholesterol ester to liver via SR-B1 receptor or transport it to steroidogenic tissues or acted upon by hepatic lipase or endothelial Lipase .
- Thus HDL_3 is reformed and free apo A-1 is released.
- Free apo A-I forms poorly lipidated Pre β HDL.
- HDL_3 and Pre Beta HDL again carry out cholesterol efflux from tissues.
- Thus ***HDL collects excess cholesterol from tissues and transport it to liver and steroidogenic tissues***. ***This is called Reverse cholesterol transport.***

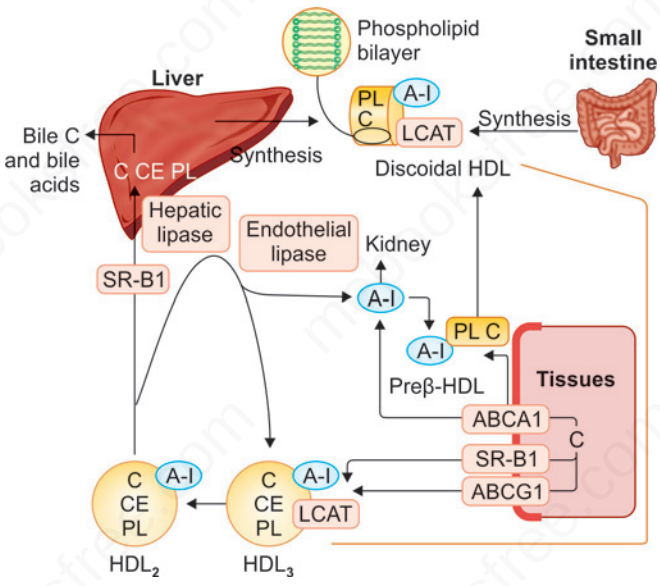


Fig. 7.27: Metabolism of HDL

Enzymes Responsible Reverse Cholesterol Transport

Lecithin Cholesterol Acyl Transferase (LCAT)

- Apo AI activates LCAT.
- Seen associated with HDL.
- Responsible for virtually all plasma Cholesteryl esters in humans.
- Esterify cholesterol in HDL.
- Cholesterol ester being nonpolar moves into the core of HDL.
- Thus create a concentration gradient and draws cholesterol from tissues.
- Thus enabling HDL to function in reverse cholesterol transport.

Cholesterol Ester Transfer Protein

- Seen associated with HDL.
- Facilitate transfer of cholesteryl ester from HDL to other lipoproteins,VLDL, IDL, LDL in exchange of Triacylglycerol.
- This relieves the product inhibition by cholesteryl ester on LCAT.
- Cholesteryl ester find its way to liver by IDL or LDL.

Receptors Responsible for Reverse Cholesterol Transport

Class B scavenger receptor B1 (SR-B1)

- Considered as HDL receptor.
- Apo A-I acts as the ligand for the receptor.
- SR-BI has dual role in HDL metabolism
- In liver and steroidogenic tissues it helps in the delivery of cholesterol and cholesterol ester to the cells from HDL.
  - In liver excess cholesterol is converted to bile acids, thus cholesterol is excreted.
  - In steroidogenic tissues, cholesterol is used for the synthesis of steroid hormones.
- Whereas from peripheral organs SR-BI facilitates cholesterol efflux to HDL particles.

ATP-binding cassette transporters

- ATP-binding cassette transporters AI (ABCAI) and ATP-binding cassette transporters G1 (ABCG1).
- Present in the extrahepatic tissues to facilitate efflux of cholesterol from peripheral tissues.
- ABCAI preferentially transport cholesterol to poorly lipidated Pre β HDL or apo AI
- ABCGI transport cholesterol to HDL.

HDL fractions

Nascent HDL or Discoidal HDL

- Contain phospholipid bilayer, cholesterol and apo AI
- Spherical HDL or HDL_3
- Contain cholesterol, cholestetryl ester, phospholipid, Apo A I, LCAT

Spherical less dense HDL or HDL_2

- Contain cholesterol, cholesteryl ester, phospholipid, Apo AI

Prebeta HDL

- Most potent HDL in efflux of cholesterol from tissues.
- Contain apo AI, cholesterol and phospholipid.
- ABCAI preferentially transfer cholesterol to Prebeta HDL.

HDL Cycle

- The interchange of HDL2 and HDL3 is called HDL Cycle.

Transport of Cholesterol between Tissues

- Dietary cholesterol is incorporated into Chylomicrons.
- 95% of Chylomicron cholesterol is delivered to liver in Chylomicron remnant.
- The cholesterol is secreted from liver in VLDL.
- Most of the cholesterol secreted in VLDL is retained in IDL and ultimately LDL.
- LDL cholesterol is taken up by liver and extrahepatic tissues.

Cholesterol Balance in the Tissues

The factors that increase the Cholesterol in the tissues

- Uptake of cholesterol-containing lipoproteins by receptors.
- Cholesterol synthesis.
- Hydrolysis of cholesteryl esters by the enzyme cholesteryl ester hydrolase.

The factors that decrease the Cholesterol in the tissues

- Efflux of cholesterol from the membrane to HDL via the ABCA1, ABCG1, or SR-B1.
- Esterification of cholesterol by ACAT (acyl-CoA: cholesterol acyltransferase).
- Utilization of cholesterol for synthesis of other steroids, such as hormones, or bile acids in the liver.

DISORDERS OF LIPOPROTEIN METABOLISM (DYSLIPOPROTEINEMIAS)

Hyperlipoproteinemias

Fredrickson Classification of Hyperlipoproteinemia

Frederickson and Levy classified hyperlipoproteinemias according to the lipoprotein particles that accumulate in the blood.

Frederick-son Type	Nomenclature	Molecular Defect	Genetic Trans-mission	Estimated Incidence
Type I	Familial Chylomicronemia Syndrome (FCS)	Lipoprotein Lipase or Apo CII	AR	1/1000000
Type IIa	Familial Hypercholesterolemia (FH)	LDL receptor	AD	1/250-1/500 (Most common)
	Familial Defective Apo B (FDB) Autosomal Dominant Hypercholesterolemia Type II (ADH Type II)	Apo B100	AD	<1/1500
	Autosomal Dominant Hypercholesterolemia Type III (ADH Type III)	PCS K9	AD	<1/1000000
	Autosomal Recessive Hypercholesterolemia (ARH)	LDL Receptor Adapter Protein (LDLRAP)	AR	<1/1000000
	Sitosterolemia	ABCG5 or ABCH8	AR	<1/1000000
Type II b	Familial Combined Hyperlipidemia (FCHL)	-----	AR	<1/1000000
Type III	Familial Dysbeta lipoproteinemia (FDBL)	Apo E	AR	1/10000
Type IV	Familial Hypertriglyceridemia (FHTG)	Apo A-V	AR	<1/1000000
Type V	Familial Hypertriglyceridemia (FHTG)	Apo A-V and GPIHBP 1	AR	<1/1000000

Lipoproteins accumulated and clinical presentation of different hyperlipoproteinemias

Phenotype	I	IIa	IIb	III	IV	V
Lipoprotein, elevated	Chylomicrons (predominant) VLDL	LDL	LDL and VLDL	Chylomicron and VLDL remnants	VLDL	Chylomicrons and VLDL
Triglycerides	↑↑↑	N	↑	↑↑	↑↑	↑↑↑

Contd...

Contd...

Phenotype	I	IIa	IIb	III	IV	V
Cholesterol (total)	↑	↑↑↑	↑↑	↑↑	N/↑	↑↑
LDL-cholesterol	↓	↑↑↑	↑↑	↓	↓	↓
HDL-cholesterol	↓↓↓	N/↓	↓	N	↓↓	↓↓↓
Plasma appearance	Lactescent	Clear	Clear	Turbid	Turbid	Lactescent
Xanthomas	Eruptive	Tendon, tuberous	None	Palmar, tuberoeruptive	None	Eruptive
Pancreatitis	+++	0	0	0	0	+++
Coronary atherosclerosis	0	+++	+++	+++	+/–	+/–
Peripheral atherosclerosis	0	+	+	++	+/–	+/–

Primary Hyperlipoproteinemias Causing Hypertriglyceridemia

Familial Chylomicronemia Syndrome (Type I Hyperlipoproteinemia)

Biochemical abnormalities

- Lipoprotein Lipase (LPL) or Apo CII defect.
- Lipoprotein Lipase is required for hydrolysis of TGs in Chylomicrons and VLDL. Apo CII is the cofactor for Lipoprotein Lipase.
- Lipoprotein accumulated is Chylomicron and VLDL, but chylomicron predominates.
- Fasting Triglycerides is > 1000 mg/dL.
- Fasting Cholesterol is elevated to a lesser degree.

Clinical presentation

- Present in childhood with recurrent abdominal pain due to acute pancreatitis
- On fundoscopic examination opalescent retinal blood vessels (lipemia retinalis)
- Lactescent plasma
- Eruptive xanthoma (small yellowish white papules appear in clusters on backs, buttocks, extensor surfaces of arms and legs. These are painless skin lesions may become pruritic)
- Hepatosplenomegaly
- Premature CHD is not a feature of FCS.

Diagnosis

Assaying triglyceride lipolytic activity in post heparin plasma (IV heparin injection to release the endothelial-bound LPL)

Observation

- LPL activity is profoundly reduced in both LPL and apoC-II deficiency
- In patients with apoC-II deficiency, it normalizes after the addition of normal plasma (providing a source of apoC-II).

Molecular sequencing of gene

New advances in treatment of Familial Chylomicronemia Syndrome

A gene therapy approach-Alipogene tiparvovec

- Multiple intramuscular injection of an adeno associated viral vector encoding again of function LPL variant, leading to skeletal myocyte expression of LPL.
- Approved in Europe.

Familial Hypertriglyceridemia (FHTG) (Type IV and V Hyperlipoproteinemia)

Biochemical abnormalities

Apo A-V deficiency

- Apo A-V facilitate association of VLDL and chylomicrons with LPL.
- Loss of function mutation of Apo A-V causes accumulation of Chylomicrons and VLDL.

GPIHBP-I deficiency

- GPIHBP-I is Glycosylated Phosphatidyl Inositol HDL Binding Protein-I
- Mutation in GPIHBP-I causes defect in transport of LPL to Vascular endothelium.
- Chylomicron is elevated.
- Triglycerides are also markedly elevated.

Primary Hyperlipoproteinemias Causing Hypercholesterolemia

Familial Hypercholesterolemia (FH)

Also known as Autosomal Dominant Hypercholesterolemia Type I (ADH Type I)

Biochemical abnormalities

- Loss of function mutation in LDL receptor gene.
- Can be
  - Homozygous or receptor negative called as homozygous FH
  - Heterozygous or receptor defective.

- Reduced clearance of LDL from circulation.
- As LDL receptor is responsible for clearance of IDL, LDL production from IDL is also increased.
- So lipoprotein accumulated is LDL and lipid accumulated is Cholesterol.
- LDL-Cholesterol >400-1000 mg/dL in homozygous cases.
- Triglycerides are usually normal.

Clinical presentation

- Family history of premature CHD
- Corneal arcus
- Plasma is clear
- No pancreatitis
- Tendon xanthomas particularly dorsum of hands and Achilles tendon
- Increased risk of cardiovascular disease.

Diagnosis

- No definitive diagnosis
- LDL receptor assay
- LDL receptor gene sequencing

Recent advances in the treatment of Homozygous Familial Hypercholesterolemia (FH)

Two orphan drug developed are

1. *Lomitapide:* Small molecule inhibitor of microsomal triglyceride transfer protein, which decreases the production of VLDL.Thereby LDL production from VLDL.
2. *Mipomersen:* Antisense oligonucleotide to apo B.

Familial Defective apo B100 (FDB)

Also known as Autosomal Dominant Hypercholesterolemia Type II (ADH Type II)

Biochemical abnormalities

- Mutation in gene encoding apo B100, specifically in the LDL receptor–binding domain of apo B-100.

Clinical presentation

- Almost like heterozygous FH.
- Plasma level of LDL-C is lower than heterozygous FH.
- This is because, IDL clearance is not impaired unlike FH as apo B100 binding domain of LDL receptor is only affected.

Diagnosis

- Sequencing receptor binding region of apo B gene.

Autosomal Dominant Hypercholesterolemia Type III (ADH Type III)

Biochemical abnormalities

- Gain of function mutation of PCSK9.
- PCSK9 is a secreted protein that binds to the LDL receptor and is redirected to the lysosome and degraded.
- So in ADH 3 there is accelerated degradation of LDL receptor.
- So LDL is not cleared .
- Clinical presentation is similar to FH.

Autosomal Recessive Hypercholesterolemia (ARH)

Biochemical abnormalities

- Due to mutations in a protein LDL Receptor adaptor protein, LDLRAP involved in LDL receptor–mediated endocytosis in the liver.
- In the absence of LDLRAP, LDL binds to the LDL receptor but the lipoprotein-receptor complex fails to be internalized.
- Clinical presentation in ARH is similar to FH.

Sitosterolemia

Biochemical abnormalities

- Mutations ATP-binding cassette (ABC) half transporter family, ABCG5 and ABCG8, expressed in enterocytes and hepatocytes which pumps plant sterols such as sitosterol and campesterol, and animal sterols, predominantly cholesterol, into the gut lumen and into the bile.
- Hence intestinal absorption of sterols is increased and biliary excretion of the sterols is reduced, resulting in increased plasma and tissue levels of both plant sterols and cholesterol.
- Increase in hepatic sterol level results in transcriptional suppression of the expression of LDL receptors, which results in reduced uptake of LDL and substantially increased LDL-C.

Clinical presentation

- Usual presentation of Familial Hypercholesterolemia like tendon xanthoma, premature atherosclerotic cardiovascular disease.
- Anisocytosis, poikilocytosis of erythrocytes, megathrombocytes due to incorporation of plant sterols into cell membrane
- Episodes of hemolysis and splenomegaly are distinctive features
- Severe hypercholesterolemia not responding to statins but dramatic response to dietary therapy or ezetimibe.

Diagnosis

- Substantial increase in plant sterols level.

Treatment

- Bile acid sequestrants
- Cholesterol absorption inhibitors.

Primary Hyperlipoproteinemias Causing Both Hypertriglyceridemia and Hypercholesterolemia

Type III Hyperlipoproteinemia

Familial Dysbetalipoproteinemia (FDBL) Or Familial Broad Beta Disease or Remnant Removal Disease

Autosomal Recessive

Biochemical abnormalities

- Due to genetic variations in apoE, especially apo E2 that interfere with its ability to bind lipoprotein receptors.
- Due to apo E gene mutation.

Apo E gene Polymorphism

- *APOE* gene is polymorphic in sequence, resulting in the expression of three common isoforms
- apoE3, which is the most common; and apoE2 and apoE4
- ApoE2 has a lower affinity for the LDL receptor; therefore, chylomicron and VLDL remnants containing apoE2 are removed from plasma at a slower rate
- Individuals who are homozygous for the E2 allele (the E2/E2 genotype) comprise the most common subset of patients with FDBL.
- Patients with apoE4 have an increased incidence of late-onset Alzheimer's disease.

Clinical presentation

- Patients with FDBL usually present in adulthood with incidental hyperlipidemia.
- Premature coronary disease, or peripheral vascular disease.
- Lipoprotein elevated is Chylomicron and VLDL remnant.
- Lipid elevated is Cholesterol and Triacylglycerol.

Xanthomas in FDBL

- Two distinctive types of xanthomas, tuberoeruptive and palmar, are seen in FDBL patients.
- **Tuberoeruptive xanthomas** begin as clusters of small papules on the elbows, knees, or buttocks and can grow to the size of small grapes.
- **Palmar xanthomas** (alternatively called xanthomata striata palmaris) are orange-yellow discolorations of the creases in the palms and wrists.
- Both these xanthomas are virtually pathognomonic of FDBL.

Diagnosis

- Very high level of remnant lipoprotein
- Lipoprotein electrophoresis-Broad beta band
- Apo E genotyping.

Hypolipoproteinemias

Abetalipoproteinemia

Autosomal recessive disease

Biochemical defect

- Loss-of-function mutations in the gene encoding microsomal triglyceride transfer protein (MTP) the gene name MTTP
- MTP transfers lipids to nascent chylomicrons and VLDLs in the intestine and liver, respectively.
- So Chylomicron^{Q}, VLDL^{Q} and hence LDL^{Q} not produced.
- Plasma levels of cholesterol and triglyceride are extremely low in this disorder.
- Chylomicrons, VLDLs, LDLs, and apo B are undetectable in plasma.

Clinical presentation

- Presents in early childhood with diarrhea and failure to thrive due to fat malabsorption
- Neurologic manifestations—loss of deep-tendon reflexes, followed by decreased distal lower extremity vibratory and proprioceptive sense, dysmetria, ataxia, and the development of a spastic gait
- Progressive pigmented retinopathy.
- Acanthocytes
- Marked deficient in vitamin E and are also mildly to moderately deficient in vitamins A and K (Due to defective fat absorption and hence the fat soluble vitamins.)^{Q}

Treatment consists of a low-fat, high-caloric, vitamin-enriched diet accompanied by large supplemental doses of vitamin E.

Vitamin deficiency in Abetalipoproteinemia^{Q}

- Most clinical manifestations of abetalipoproteinemia result from defects in the absorption and transport of fat-soluble vitamins.
- Vitamin E and retinyl esters are normally transported from enterocytes to the liver by chylomicrons.
- Vitamin E is dependent on VLDL for transport out of the liver and into the circulation.
- As apo B containing lipoproteins are not formed these patients, there is, marked deficiency of Vitamin E, mild to moderate deficiency of Vitamin A and K.
- Bleeding manifestation due to defective absorption of fat and hence fat soluble vitamins.

Tangier Disease

Autosomal codominant

Biochemical defect

- Mutations in the gene encoding ABCA1, a cellular transporter that facilitates efflux of unesterified cholesterol and phospholipids from cells to apo A-I.
- In the absence of ABCA1, the nascent HDL, poorly lipidated.

- ApoA-I is immediately cleared from the circulation.
- Extremely low circulating plasma levels of HDL-C and apo A-I.
- Cholesterol accumulates in the reticuloendothelial system.

Clinical presentation

- Hepatosplenomegaly
- Enlarged, grayish yellow or orange tonsils (Pathognomonic Clinical feature)
- An intermittent peripheral neuropathy (mononeuritis multiplex)
- Probably associated with high incidence of Premature atherosclerotic disease, although association is not robust as expected.

LCAT Deficiency

Two genetic forms of LCAT deficiency.

Complete deficiency (*also called classic LCAT deficiency*) or Norum's Disease

Clinical Presentation

- Progressive corneal opacification due to the deposition of free cholesterol in the cornea.
- Hemolytic anemia
- Progress to ESRD

Biochemical abnormalities

- Very low plasma levels of HDL-C
- Rise in free cholesterol
- Rise in lecithin
- Fall in Lysolecithin, Cholesterol ester, HDL.

Partial LCAT deficiency (also called fish-eye disease)

Same features as classic LCAT deficiency, but no Hemolytic Anemia and do not progress to ESRD Diagnosis.

- Assay Plasma LCAT activity.
- Sequencing LCAT gene.

Secondary Hyperlipoproteinemia

Secondary causes of increased VLDL production

Cause	Biochemical defect
High Carbohydrate diet	Excess dietary carbohydrate is converted to fatty acid in the liver, esterified to form Triacyl glycerols, secreted from liver as VLDL
Alcohol	The most common effect of alcohol is to increase plasma triglyceride levels. • Alcohol consumption inhibit the hepatic oxidation of free fatty acids, which then promote **hepatic triglyceride synthesis and VLDL secretion**

Contd...

Contd...

Cause	Biochemical defect
Obesity and Insulin resistance	The increase in adipocyte mass and accompanying decreased insulin sensitivity associated with obesity has multiple effects on lipid metabolism. • More free fatty acids are delivered from the expanded adipose tissue to the liver. • The increased insulin levels promote fatty acid synthesis in the liver. • Increased Insulin resistance causes reduced LPL activity which causes reduced clearance of Chylomicron and VLDL. This results in hypertriglyceridemia.
Nephrotic Syndrome	• Classic cause of excessive VLDL production. • Exact cause unknown but attributed to hypoalbuminemia leading to increased hepatic protein synthesis. • Increased VLDL production, hence increased LDL from VLDL.
Cushings Syndrome	Exogenous or endogenous glucocorticoids lead to increased VLDL production.

Secondary causes of reduced hepatic uptake of lipoproteins

Cause	Biochemical defects
Hypothyroidism	• Thyroid hormone increases the hepatic expression of LDL receptor. • So hypothyroidism reduction in hepatic LDL receptor, reduced clearance of LDL. • Elevated LDL-C.
Chronic Kidney disease	• Accumulation of VLDL, and remnant lipoproteins and mild hypertriglyceridemia. • Due to reduced TG lipolysis and remnant lipoprotein clearance.

Liver disorders and secondary hyperlipoproteinemias

- Liver is the principal site of formation and clearance of lipoproteins
- So liver diseases can affect plasma lipid levels in a variety of ways.

Hepatitis due to infection, drugs, or alcohol

- Associated with increased VLDL synthesis and mild to moderate hypertriglyceridemia.
- Severe hepatitis and liver failure
- Associated with dramatic reductions in plasma cholesterol and triglycerides due to reduced lipoprotein biosynthetic capacity.

Cholestasis

- Associated with hypercholesterolemia, which can be very severe.
- A major pathway by which cholesterol is excreted from the body is via secretion into bile, either directly or after conversion to bile acids, and cholestasis blocks this critical excretory pathway.

In cholestasis, free cholesterol, coupled with phospholipids, is secreted into the plasma as a constituent of a lamellar particle called LP-X.

Estrogen and Secondary Hyperlipoproteinemia

- Estrogen administration is associated with increased VLDL and HDL synthesis.
- Resulting in elevated plasma levels of both triglycerides and HDL-C.
- This lipoprotein pattern is distinctive since the levels of plasma triglyceride and HDL-C are typically inversely related

Calculation of Lipid Fractions

- In fasting,cholesterol is carried primarily on three lipoproteins-the VLDL, LDL, and HDL
  - Total Cholesterol = HDL Cholesterol + VLDL Cholesterol + LDL Cholesterol
- VLDL contains five times as much triglyceride by weight as cholesterol.
  - So VLDL Cholesterol = Triglycerides/5
- This formula will work when triglyceride level < 400 mg/dL
- Friedwalds formula for LDL-cholesterol (all values in mg/dL)[a]

$$\text{LDL Cholesterol} = \text{Total Cholesterol-HDL cholesterol} - \frac{\text{Triglycerides}}{5}$$

PHARMACOLOGIC TREATMENT OF LIPOPROTEIN DISORDERS

For severe Hypertriglyceridemia

Fibric Acid Derivatives (Fibrates)

Fibric acid derivatives are agonists of PPARa, a nuclear receptor involved in the regulation of lipid metabolism.

- Stimulate LPL activity (enhancing triglyceride hydrolysis)
- Reduce apoC-III synthesis (enhancing lipoprotein remnant clearance)
- Promote beta-oxidation of fatty acids
- Reduce VLDL triglyceride production.

Fibrates are the most effective drugs available for reducing triglyceride levels and also raise HDL-C levels modestly

- Gemfibrosil
- Fenofibrate.

Omega 3 Fatty Acids (Fish Oils)

The most widely used ω-3 PUFAs for the treatment of hyperlipidemias

- Eicosapentaenoic acid (EPA)
- Docosa hexanoic acid (DHA).

Nicotinic Acid (Niacin)

Niacin suppress lipolysis in the adipocytes due to its effect on GPR109A and also has other poorly understood mechanisms on hepatic lipid metabolism.

For Hypercholesterolemia

HMG-CoA Reductase Inhibitors (Statins)

HMG-CoA reductase is a key enzyme in cholesterol biosynthesis, and inhibition of this enzyme decreases cholesterol synthesis.

Cholesterol Absorption Inhibitors

- Ezetimibe

Cholesterol absorption inhibitor that binds directly to and inhibits NPC1L1 and blocks the intestinal absorption of cholesterol.

Bile Acid Sequestrants (Resins)

- **Cholestyramine, Colestipol, colesevelam**
- Bile acid sequestrants bind bile acids in the intestine and promote their excretion rather than reabsorption in the ileum.
- To maintain the bile acid pool size, the liver diverts cholesterol to bile acid synthesis.
- The decreased hepatic intracellular cholesterol content results in upregulation of the LDL receptor and enhanced LDL clearance from the plasma.

LDL Apharesis

- Treatment of hypercholesterolemia
- Patients blood is passed through a column that selectively removes the LDL.

Specialized drugs for homozygous Familial Hypercholesterolemia.

- MTP inhibitor-Lomitapide
- Apo B inhibitor-Mipomersen.

ATP III Guidelines for Ideal Level of LDL, HDL and Cholesterol

Biochemical parameter	Values mg/dL	Risk
LDL-Cholesterol	<100	Optimum
	100–129	Near or above Optimum
	130–159	Borderline high
	160–189	High
	> 190	Very High
Total-Cholesterol	< 200	Desirable
	200–239	Borderline High
	> 240	High
HDL-Cholesterol	< 40	Low
	> 60	High

Predictors of Coronary Artery Diseases

- hs CRP
- Total Cholesterol/HDL ratio
- Apo B/Apo A ratio
- LDL
- Non HDL Cholesterols
- HDL-Cholesterol

REVIEW QUESTIONS

TAG Synthesis

1. True about ac.etyl CoA: (PGI Nov 2011)

a. Precursor for synthesis of cholesterol and other steroids
b. Form Ketone bodies
c. Starting material for synthesis of fatty acid
d. Arise from Glycolysis

Ans. a, b, c, d. *(Ref: Harper 30/e p226, 273, 234)*

Fates of Acetyl CoA

- Synthesis of Cholesterol and other steroids.
- Synthesis of Fatty acid.
- Synthesis of Ketone Bodies.
- Enter in to TCA Cycle.

2. Regarding synthesis of triacylglycerol in adipose tissue, all of the following are true except: (AI 07)

a. Synthesis from Dihydroxy acetone phosphate
b. Enzyme Glycerol kinase plays an important role
c. Enzyme Glycerol 3 phosphate dehydrogenase plays an important role
d. Phosphatidate is hydrolyzed

Ans. b. Enzyme Glycerol kinase plays an important role

In Muscle and Adipose Tissue

- Glycerol Kinase is absent in muscle and white adipose tissue.
- Glycerol 3 Phosphate is formed from Dihydroxy Acetone Phosphate, an intermediate in Glycolysis.

3. The storage Triacylglycerol are hydrolyzed by: (JIPMER 2012)

A. Pancreatic Lipase
B. Lipoprotein Lipase
C. Lysosomal Lipase
D. Hormone sensitive Lipase

Ans. d. Hormone sensitive lipase *(Ref: Harper 30/e p263)*

- Pancreatic lipase to hydrolyze dietary TGs
- Lipoprotein lipase to hydrolyze TGs in lipoprotein in the blood
- Lysosomal hydrolase to act on TGs in lysosomes
- Hormone sensitive lipase hydrolyze stored TGs in adipose tissue.

Fatty acid Synthesis

4. Which of the following is not a part of Fatty Acid Synthase Complex? (AIIMS Nov 2013)

a. Ketoacyl Reductase
b. Enoyl Reductase
c. Acetyl CoA Carboxylase
d. Keto Acyl Synthase

Ans. c. Acetyl CoA Carboxylase *(Ref: Harper 30/e p234)*

Fatty Acid Synthase (FAS) Multienzyme Complex

- The complex is a homodimer of two identical polypeptide monomers in which **six enzyme activities and the acyl carrier protein (ACP)**
- ACP contains the vitamin **Pantothenic acid** in the form of 4′-phosphopantetheine
- X-ray crystallography of the three-dimensional structure, shown that the complex is arranged in an X shape
- Acetyl CoA carboxylase is not a part FAS Complex.º

5. Mitochondria is involved in A/E: (AI 2012)

a. Fatty Acid Synthesis
b. DNA Synthesis
c. Fatty Acid Oxidation
d. Protein Synthesis

Ans. a. Fatty Acid Synthesis *(Ref: Harper 30/e p233)*

Mitochondria is involved in mitochondrial DNA Synthesis and Protein Synthesis (described in Molecular genetics chapter).

De Novo Fatty Acid Synthesis (Lipogenesis)

- Major contribution to discovery by Feodor Lynen, hence the pathway is otherwise called **Lynen's Spiral**
- Site: Liver, kidney, brain, lung, lactating mammary gland, and adipose tissue
- Organelle: By an **extra mitochondrial system** [in the cytosol]

Cofactor requirements include

- NADPH, ATP, Mn^{2+}, Biotin, and HCO_{3-} (as a source of CO_2)
- Acetyl-CoA is the principal building block of Fatty Acid.

6. Fatty acid synthase complex contain the following enzymes except: (Kerala 2010)

a. Enoylreductase
b. Ketoacylreductase
c. Acetyl CoA carboxylase
d. Dehydratase

Ans. c. Acetyl CoA Carboxylase *(Ref: Harper 30/e p234)*

Six enzyme activities of Fatty Acid Synthase Complex are

- Ketoacyl Synthase
- Malonyl-Acetyl Transacylase
- Hydratase
- Enoyl Reductase
- Ketoacyl Reductase
- Thioesterase (Deacylase).

7. NADPH is required for: (AI 1998)

a. Gluconeogenesis
b. Glycolysis
c. Fatty acid synthesis
d. Glycogenolysis

Ans. c. Fatty acid synthesis *(Ref: Harper 30/e p235)*

8. The first step in fatty acid synthesis involves: (AI 1996)

a. Acetyl CoA carboxylase
b. B – Hydroxyl CoA dehydrogenase
c. Acetyl dehydrogenase
d. Pyruvate kinase

Ans. a. Acetyl CoA Carboxylase *(Ref: Harper 30/e p235)*

Fatty Acid Synthesis

By two enzyme system

- Acetyl CoA Carboxylase
- Fatty Acid Synthase Complex

Acetyl CoA Carboxylase

- Converts Acetyl CoA (2C) to Malonyl CoA in the presence of ATP.
- Acetyl-CoA carboxylase has a requirement for the B vitamin biotin.
- It is a multienzyme protein.
- This is the rate limiting step^{Q} in the Fatty Acid Synthesis.
- Acetyl CoA carboxylase is not a part FAS Complex.^{Q}

9. In fatty acid synthesis CO_2 loss occurs in which step: (PGI Dec 2006)

a. Hydration
b. Dehydration
c. Condensation reaction
d. Reduction

Ans. c. Condensation reaction *(Ref: Harper 30/e p235)*

Reactions of Fatty Acid Synthase Complex

By three stages

- Condensation
- Reduction
- Release of Fatty acid

Condensation Reactions

- **Malonyl Acetyl Transacylase**
  - A priming molecule of acetyl-CoA combines with a cysteine—SH group.
  - While malonyl-CoA combines with the adjacent —SH on the 4′-phosphopantetheine of ACP of the other monomer.
  - These reactions are catalyzed by **malonyl** acetyl **transacylase**, to form **acetyl (acyl)-malonyl enzyme**
- **Ketoacyl Synthase**
  - The acetyl group attacks the methylene group of the malonyl residue, catalyzed by 3-ketoacyl synthase, and **liberates CO_2**, forming 3-ketoacyl enzyme (acetoacetyl enzyme) (reaction 2), freeing the cysteine—SH group.

Reduction reactions

- **Ketoacyl Reductase**
  - The 3-ketoacyl group is reduced to Hydroxyacyl group by Ketoacyl Reductase
- **Hydratase**
  - Hydroxyacyl group is dehydrated to Unsaturated Acyl (Enoyl) group by Hydratase
- **Enoyl Reductase**
  - Unsaturated Acyl (Enoyl) group is reduced to Acyl group by Enoyl Reductase.

Releasing of Fatty Acid

These reactions of Condensation and reduction repeated several times till the desired acyl group is assembled on the enzyme.

Thioesterase

- Fatty acid (Acyl group) is liberated from the enzyme complex by the activity of the sixth enzyme in the complex, thioesterase (deacylase).

10. Carbon atoms added in fatty acid synthesis: (AIIMS Nov 91)

a. 2 in Ist cycle and 4 in IInd cycle
b. 4 in Ist cycle and 2 in IInd cycle
c. 2 in Ist cycle and 2 in IInd cycle
d. 4 in Ist cycle and 4 in IInd cycle

Ans. b. 4 in Ist cycle and 2 in IInd cycle

(Ref: Harper 30/e p235)

- First Cycle of FAS Complex 2 C from Acetyl CoA condenses with 2 Carbon atom of Malonyl CoA, by liberating 1 CO_2, So 4 Carbon atoms added.
- In the second cycle to the existing 4 Carbon fatty acyl residue, 2 Carbon is added by Ketoacyl Synthase.

11. True about mitochondrial chain elongation of fatty acid is/are: (PGI June 1999)

a. Operates under anaerobic conditions
b. Operates aerobically
c. Common pathway
d. Not a common pathway
e. Pyridoxal-Phosphate and NADPH is required

Ans. b. Operates aerobically, **d.** Not a common pathway

Elongation of Fatty Acid Chains

- Occurs in the Endoplasmic Reticulum (the 'microsomal system') and some in mitochondria also.
- By Fatty Acid Elongase system
- Elongates saturated and unsaturated fatty acyl-CoAs (from C10 upward) by two carbons
- Malonyl CoA donates 2 Carbon atoms in stepwise manner
- In the same manner as Fatty acid Synthase Complex in the Cytosol
- NADPH is required at the two reductase step
- Elongation reaction are **particularly increased in brain during myelination to provide C22 and C24 fatty acids for sphingolipids. (So not common)**.

12. PAN-SH site of fatty acid synthase complex accepts: (PGI Dec 2000, AIIMS Dec 94)

a. Acetyl CoA
b. Malonyl CoA
c. Propionyl CoA
d. All

Ans. b. Malonyl CoA *(Ref: Harper 30/e p235)*

- Cys–SH group accept Acetyl CoA
- Pan-SH group accepts Malonyl CoA.

13. In which organelle(s) of hepatocyte, the elongation of long chain fatty acid takes place: (PGI Dec 08)

a. Endoplasmic reticulum
b. Golgi body
c. Mitochondria
d. Lysosomes
e. Ribosome

Ans. a. Endoplasmic reticulum, **c.** Mitochondria *(Ref: Harper 30/e p236)*

14. Acetyl CoA acts as a substrate for all the enzymes except: (AIIMS May 03)

a. HMG-CoA synthase
b. Malic enzyme
c. Malonyl CoA synthetase
d. Fatty acid synthetase

Ans. b. Malic enzyme *(Ref: Harper 30/e p236 fig. 23-4)*

- Malic Enzyme converts Malate to Pyruvate by liberating CO_2.

15. Acetyl CoA Carboxylase is activated by: (NBE pattern Q)

a. Malonyl CoA
b. Citrate
c. Palmitoyl CoA
d. Acetoacetate

Ans. b. Citrate *(Ref: Harper 30/e p237)*

Allosteric regulation of Acetyl CoA carboxylase

- Positive Allosteric regulation or Allosteric Activation of Acetyl CoA Carboxylase by Citrate[Q]
- **Palmitoyl CoA is an inhibitor of Acetyl CoA Carboxylase**.

Oxidation of Fatty Acid and Disorders

16. Number of ATP formed by oxidation of one molecule of palmitic acid (16 c): (Kerala 2009)

a. 146
b. 106
c. 135
d. 34

Ans. b. 106 *(Ref: Harper 30/e p226)*

Energetics of Beta Oxidation

The number of ATPs obtained depends on the number of Carbon atoms in the fatty acid from Beta oxidation of Palmitic acid (C-16)

First calculate how many cycles of beta oxidation [(n/2)–1] where n = no of carbon atoms

- In case of palmitic acid, 7 cycles of beta oxidation
- From one cycle of beta oxidation
- 1 FADH2 = 5 ATPs
- 1 NADH = 2.5 ATPs
- Total = 4 ATPs
- **From 7 cycles of beta** oxidation
- 7 × No of ATPs from 1 cycle
- 7 × 4 = **28 ATPs**

In case of Palmitic Acid (16/2) = 8 Acetyl CoA

- From one Acetyl CoA by TCA Cycle 10 ATPs
- From 8 Acetyl CoA

- 8 × 10 = **80 ATPs**
- **Total ATPs from Palmitic Acid = 28 + 80 = 108**
- ***2 ATPs utilized for initial activation of Fatty Acid***
- ***So net ATPs from Palmitic Acid = 108–2 = 106 ATPs.***

17. Beta Oxidation in Peroxisome generate: (NBE Pattern Q)

a. NADPH
b. H_2O_2
c. Long Chain fatty acid
d. $FADH_2$

Ans. b. H_2O_2 *(Ref: Harper 30/e p226)*

A modified form of β-oxidation is found in peroxisomes and leads to the formation of acetyl-CoA and H_2O_2 (from the flavoprotein-linked dehydrogenase step), which is broken down by catalase. Thus, this dehydrogenation in peroxisomes is not linked directly to phosphorylation and the generation of ATP. The system facilitates the oxidation of very long chain fatty acids (e.g. C_{20}, C_{22}). The enzymes in peroxisomes do not attack shorter chain fatty acids; the β-oxidation sequence ends at octanoyl-CoA.

18. All are features of Refsum's disease except: (PGI Nov 2014)

a. Deficiency of alpha hydroxylase
b. Defect of beta oxidation
c. Accumulation of Phytanic acid
d. Peripheral neuropathy
e. Treated by removing Phytanic acid precursors from diet

Ans. a, c, d, e.

Refsum's Disease

- Defect in **Alpha oxidation of Phytanic Acid (Phytanic Acid Oxidase) (phytanoyl CoA hydroxylase**) in the Peroxisome.
- The manifestation of classic Refsum's Disease includes impaired vision from retinitis pigmentosa, ichthyosis, **peripheral neuropathy**, ataxia, and, occasionally, cardiac arrhythmias.
- Classic Refsum disease often does not manifest until young adulthood, but visual disturbances such as night blindness, ichthyosis, and peripheral neuropathy may already be present in childhood and adolescence.
- **Restrict dietary dairy products** and Green Leafy Vegetables.

19. Enzyme defect in Refsum's disease: (NBE pattern Q)

a. Phytanoyl Alpha Oxidase
b. Acyl CoA Dehydrogenase
c. Thiolase
d. Thiokinase

Ans. a. Phytanoyl Alpha Oxidase

(Ref: Dinesh Puri 3/e p210; Nelson 20/e Chapter Defects in metabolism of lipids)

20. Adrenoleukodystrophy is associated with: (CMC Vellore 2014)

a. Accumulation of very long chain fatty acids
b. Accumulation of medium chain fatty acid
c. Increased Plasmalogen
d. Decreased Pipecolic Acid

Ans. a. Accumulation of Very Long Chain Fatty Acid

Abnormal laboratory findings common to disorders of peroxisome biogenesis

- Peroxisomes absent to reduced in number
- Catalase in cytosol
- Deficient synthesis and reduced tissue levels of plasmalogens
- Defective oxidation and abnormal accumulation of very long chain fatty acids
- Deficient oxidation and age-dependent accumulation of phytanic acid
- Defects in certain steps of bile acid formation and accumulation of bile acid intermediates
- Defects in oxidation and accumulation of l-pipecolic acid
- Increased urinary excretion of dicarboxylic acids

21. β-oxidation of palmitic acid yields: (PGI Dec 05)

a. 3 acetyl CoA
b. 129 ATP net
c. 131 ATP net
d. 16 acetyl CoA
e. 96 ATP from citric acid cycle

Ans. b. 129 ATP net, **e.** 96 ATP from citric acid cycle

This question is based on older calculation, i.e. 1 NADH = 3 ATPs, 1 FADH2 = 2 ATPS

- Palmitic acid oxidation has 7 cycles and 8 Acetyl CoA
- I cycle of beta oxidation yield 3 + 2 = 5 ATPs
- 7 cycles yield 35 ATPs
- 8 Acetyl CoA by TCA cycle yield 8 × 12 = 96 ATPs
- So total ATPs = 96 + 35 = 131 ATPs
- 2 ATPs for initial activation
- So net ATPs produced from Palmitic acid = 131 – 2 = 129 ATPs
- According to new calculation
- 106 ATPs from 1 mol of Palmitic acid by beta oxidation.

22. β-oxidation in peroxisome is differentiated from that occurring in mitochondria by: (PGI June 03)

a. Acetyl CoA
b. H_2O_2 formed
c. Different enzymes are found in different site
d. NADH is required

Ans. b. H_2O_2 formed *(Ref: Harper 30/e p226)*

Oxidation of Very Long Chain Fatty Acid

- By a modified β-oxidation pathway
- For Fatty Acids >C_{20},C_{22}
- Takes place in the **Peroxisomes** till Octanoyl CoA
- Oxidation in peroxisome produces Acetyl CoA and H_2O_2 (instead of $FADH_2$)
- No ATP is generated.

Further oxidation of Acetyl CoA and Octanoyl CoA takes place in the **mitochondria**.

23. One of the following is obtained in the by beta oxidation of odd chain fatty acids: (JIPMER 2013)

a. Acetyl CoA + Acetyl CoA
b. Acetyl CoA + Propionyl CoA
c. Propionyl CoA + Propionyl CoA
d. Acetyl CoA alone

Ans. b. Acetyl CoA + Propionyl CoA *(Ref: Harper 30/e p225)*

Oxidation of Odd Chain Fatty Acid

- Takes place in the mitochondria.
- Oxidation of a Fatty Acid with an odd number of carbon atoms yields Acetyl CoA and a molecule of **Propionyl-CoA**.
- The propionyl residue from an odd-chain fatty acid is the only part of a fatty acid that is glucogenic.

Ketone Bodies

24. Which of the following organs do not utilize ketone bodies? (PGI May 2014)

a. Brain
b. RBC
c. Muscle
d. Heart
e. Liver

Ans. b. RBC, **e.** Liver

25. Ketone bodies can be utilized by all, except: (AIIMS May 2013)

a. RBC
b. Brain
c. Skeletal Muscle
d. Renal Cortex

Ans. a. RBC *(Ref: Harper 30/e p227)*

- Ketone Bodies Serve as a Fuel for Extrahepatic Tissues
- Almost all the organs utilize Ketone bodies with the exception of *Liver and RBCs.*

26. Rothera's test used for detection of: (Kerala 2010)

a. Proteins
b. Glucose
c. Fatty Acid
d. Ketones

Ans. d. Ketone Bodies

Test for Ketone Bodies

- Gerhardt's Ferric Chloride Test: Detect Acetoacetate
- Rothera's Nitroprusside Test: Detect Acetoacetate and Acetone
- None of the above tests detect Beta hydroxybutyrate.

Name of the test	Compound detected
Rothera's Test	Ketone Bodies, Branched chain Ketoacids
Hay's test	Bile salt
Liebermann Burchard reaction	Cholesterol
Salkowski's Reaction	Cholesterol

27. Which organ does not utilize ketone bodies: (AIIMS Sep 96)

a. Liver
b. Brain
c. Skeletal muscle
d. Cardiac muscle

Ans. a. Liver *(Ref: Harper 30/e p227)*

28. The immediate precursor in the formation of acetoacetate from acetyl CoA in the liver is: (PGI June 99)

a. Mevalonate
b. HMG CoA
c. Acetoacetyl CoA
d. 3-hydroxyl-butyryl CoA

Ans. b. HMG CoA *(Ref: Harper 30/e p228)*

Hydroxy-3-methylglutaryl-CoA lyase then causes acetyl-CoA to split off from the HMG-CoA, leaving free acetoacetate.

29. In a well fed state, acetyl CoA obtained from diet is least used in the synthesis of: (AI 2002)

a. Palmitoyl CoA
b. Citrate

c. Acetoacetate
d. Oxalosuccinate

Ans. c. Acetoacetate

- In well fed state fatty acid (Palmitic acid) synthesis is active
- TCA cycle also takes place, Citrate and oxalosuccinate are intermediates in TCA Cycle
- Oxalosuccinate is an intermediate in the reaction of Isocitrate Dehydrogenase of TCA Cycle
- Acetoacetate, a primary ketone body is synthesized in fasting state.

Cholesterol Synthesis

30. Common enzyme in cholesterol and Ketone body metabolism: (AI 2012)

a. HMG CoA reductase
b. HMG CoA lyase
c. HMG CoA synthase
d. Thiolase

Ans. b. HMG CoA Synthase, **d.** Thiolase
(Ref: Harper 30/e p228, 267)

Compare–cholesterol synthesis and ketone body synthesis

Characteristics	Ketone body synthesis	Cholesterol synthesis
Site	Mitochondria	Cytoplasm
HMG CoA as an intermediate	Yes	Yes
HMG CoA Synthase	Yes, the regulatory step	Yes
HMG CoA Reductase	No	Yes, the rate limiting step
HMG CoA Lyase	Yes	No
Thiolase	Yes	Yes

- Thiolase is an enzyme involved in KB synthesis. Thiolase convert Acetoacetyl CoA to Acetyl CoA.
- In Cholesterol synthesis, two mols of acetyl CoA condenses to form Acetoacetyl CoA by Thiolase.
- Cytoplasmic HMG CoA Synthase for Cholesterol synthesis.
- Mitochondrial HMG CoA Synthase for KB synthesis.

31. All are derived from cholesterol except: (Kerala 2011)

a. Vitamin D
b. Bile salt
c. Bile pigment
d. Steroid

Ans. c. Bile Pigment *(Ref: Harper 30/e p267)*

Derivatives of Cholesterol

- Bile acids
- Vitamin D
- Corticosteroids
- Sex Hormones

32. Which of the following does not have cholesterol? (Kerala 2006)

a. Vitamin D
b. Estrogen
c. Adrenaline
d. Progesterone

Ans. c. Adrenaline *(Ref: Harper 30/e p267)*

Specialized Products of Cholesterol

- Bile Acids [Excretory form of Cholesterol]
- Vitamin D
- Sex Hormones
- Corticosteroids

33. Which coenzyme act as reducing agent in anabolic reaction?

a. FADH2
b. FMNH2
c. NADPH
d. NADH

Ans. c. NADPH

NADPH helps in the reductive biosynthesis of fatty acids, Cholesterol and steroid hormones.

34. Enzyme common for synthesis of both ketone bodies and Cholesterol: (JIPMER Nov 2015)

a. HMG CoA Reductase
b. HMG CoA Synthase
c. Acetyl CoA Carboxylase
d. HMG CoA Lyase

Ans. b. HMG CoA Synthase *(Ref: Harper 30/e p237, 267)*

Characteristics	Ketone Body Synthesis	Cholesterol Synthesis
Site	Mitochondria	Cytoplasm/Smooth Endoplasmic reticulum
HMG CoA as an intermediate	Yes	Yes
HMG CoA Synthase	Yes, the regulatory step	Yes
HMG CoA Reductase	No	Yes, the rate limiting step
HMG CoA Lyase	Yes	No

Bile Acids

35. Bile acids are derived from: (AI 1994)

a. Fatty acids
b. Cholesterol
c. Bilirubin
d. Proteins

Ans. b. Cholesterol *(Ref: Harper 30/e p267)*

- Bile acids are the excretory form of cholesterol.

36. Bile acids synthesized in liver (primary bile acids) (PGI Dec 2000)

a. Lithocolic acid
b. Cholic acid
c. Chenodeoxycholic acid
d. Deoxycholic acid
e. Taurocholic acid

Ans. b. Cholic acid, **c.** Chenodeoxycholic acid, **e.** Taurocholic acid *(Ref: Harper 30/e p273)*

Primary Bile acids—Liver

They are:

- Cholic Acid (Most abundant bile acids in mammals)
- Chenodeoxycholic Acid or Chenic acid

Secondary Bile acids—Intestine

They are:

- Deoxycholic Acid
- Lithocholic Acid

Conjugation of Primary Bile Acids

- Primary bile acids are conjugated with Glycine or Taurine.
- Conjugation takes place in liver peroxisomes.
- In humans the ratio of Glycine to Taurine conjugates is 3:1.

With Glycine	With Taurine
Glycocholic Acid	Taurocholic acid
Glycochenodeoxycholic acid	Taurochenodeoxycholic Acid.

Lipoprotein Metabolism

37. Which is the ligand for receptors present in liver for uptake of LDL: (May 2009)

a. apo E
b. apo A and apo E
c. apo E and apo B100
d. apo B100

Ans. d. apo B100 *(Ref: Harper 30/e p271)*

- LDL receptor is used for the uptake of LDL and other remnant lipoprotein (VLDL remnant)
- LDL receptor has ligand binding site for both apo E and apo B100
- For uptake of LDL apo B100 acts as the ligand for LDL receptor
- For uptake of remnant lipoproteins apo E acts as the ligand for LDL receptor.

LDL Receptor

- Function uptake of LDL and remnant lipoprotein containing apo E. Ligand for LDL receptor is apoB100 and apo E.
- Present in the Liver and extrahepatic tissues (adipose tissue, Heart etc).
- **High level of cholesterol upregulate LDL receptor, causing an increase in the uptake LDL.**
- Mechanism of uptake is **receptor mediated uptake or absorptive pinocytosis.**
- Vesicles formed during absorptive pinocytosis are derived from invaginations (pits) are coated on the cytoplasmic side with a filamentous material clathrin, hence the pits are named coated pits.
- For uptake of LDL, apo B100 acts as the ligand.

Clathrin

- Three-limbed structure (called a **triskelion**)
- With each limb being made up of one light and one heavy chain of clathrin.

38. Triglycerides are maximum in: (AIIMS May 2007)

a. Chylomicrons
b. VLDL
c. LDL
d. HDL

Ans. a. Chylomicrons *(Ref: Harper 30/e p254 Table 25-1)*

Lipo-protein	Source	Protein content (%)	Lipid Content (%)	Main lipid compo-nent	Apolipo-proteins
Chylo-microns	Intes-tine	1–2 (Mini-mum)	98–99 (Maxi-mum)	Triacylg-lycerol	A-I, A-II, A-IV, B-48, C-I, C-II, C-III, E
VLDL	Liver (intes-tine)	7–10	90–93	Triacylg-lycerol	B-100, C-I, C-II, C- III
IDL	VLDL	11	89	Triacyl-glycerol, cholesterol	B-100, E

Contd...

Contd...

Lipo-protein	Source	Protein content (%)	Lipid Content (%)	Main lipid compo-nent	Apolipo-proteins
LDL	VLDL	21	79	Choles-terol	B-100
HDL	Liver, intes-tine	32 (Maxi-mum)	68 (Mini-mum)	Phos-pholipids, Choles-terol	A-I, A-II, A-IV, C-I, C-II, C-III, D,E

39. Increased level of lipoprotein (a) predisposes to: **(AIIMS May 2007)**

a. Liver cirrhosis
b. Atherosclerosis
c. Nephritic syndrome
d. Pancreatitis

Ans. b. Atherosclerosis

(Ref: Vasudevan and Sreekumari 7/e p179)

Lp (a)

- Almost similar to LDL.
- Attached to B100 by disulphide bond.
- Strongly associated with Atherosclerosis and Myocardial infarction.
- Significant homology with Plasminogen.
- It interferes with activation of Plasminogen to plasmin.
- Hence, fibrin clot is no lysed.
- Susceptible to Intravascular thrombosis.

Lpx

- Cholesterol is excreted as bile acids in the bile.
- In cholestasis, cholesterol combines with Phospholipid and form Lipoprotein X.
- Hence, it is an index of cholestasis.

40. Main transporter of cholesterol to peripheral tissue: **(PGI Nov 2011)**

a. HDL
b. LDL
c. VLDL
d. IDL
e. Chylomicron

Ans. b. LDL *(Ref: Harper 30/e p272, 273)*

Transport of Cholesterol between tissues

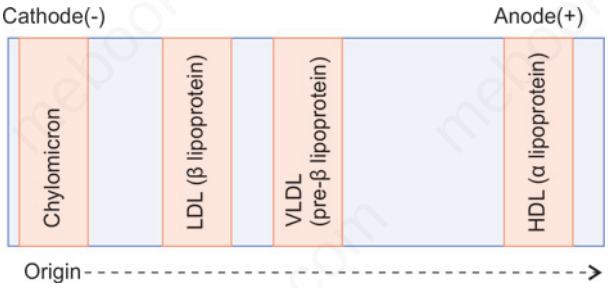
- Dietary cholesterol is incorporated into Chylomicrons.
- 95% of Chylomicron cholesterol is delivered to liver in Chylomicron remnant.
- The cholesterol is secreted from liver in VLDL.
- Most of the cholesterol secreted in VLDL is retained in IDL and ultimately LDL.
- *LDL* cholesterol is taken up by liver and extrahepatic tissues *(peripheral tissues)*.

41. Which of the following lipoproteins does not move towards charged end in electrophoresis: **(AI 2010)**

a. VLDL
b. LDL
c. HDL
d. Chylomicrons

Ans. d. Chylomicron *(Ref: Lippincott 6/e p228, fig 18.5)*

Electrophoretic Separation of Lipoproteins



Depends on the Charge (Protein content is one of the major determinants of charge) content

- Higher the protein content faster the mobility of lipoprotein in the electric field.
- Chylomicron remains at the origin
- HDL moves fastest.

From Cathode to Anode the Order of Lipoprotein

- Chylomicron
- LDL (β Lipoprotein)
- VLDL (Pre β Lipoprotein)
- IDL (Broad β Lipoprotein)
- HDL (α Lipoprotein)

42. All of the following statements about Lipoprotein Lipase are true, Except: **(AI 2009)**

a. Found in adipose tissue
b. Found in myocytes
c. Deficiency leads to hypertriacylglycerydemia.
d. Does not require CII as cofactor

Ans. d. Does not require CII as cofactor

(Ref: Harper 30/e p257)

Option a and b

- Lipoprotein lipase is not present in the myocyte and adipose tissue.
- It is actually present in the capillaries of these organs.
- But option d, definitely wrong so it is given as the answer.

Lipoprotein Lipase

- Is located on the walls of blood capillaries.
- Is anchored to the endothelium by negatively charged proteoglycan chains of heparansulfate.
- It has been found in heart, adipose tissue, spleen, lung, renal medulla, aorta, diaphragm, and lactating mammary gland, although it is not active in adult liver.
- It is not normally found in blood; however, following injection of **heparin**, lipoprotein lipase is released from its heparansulfate binding sites into the circulation.

Both **phospholipids** and **apo C-II** are required as cofactors for lipoprotein lipase activity, while **apo A-II** and **apo C-III** act as inhibitors.

Reaction Catalyzed by Lipoprotein Lipase

- Triacylglycerol is hydrolyzed progressively through a diacylglycerol to a monoacylglycerol and finally to FFA plus glycerol.
- Bulk of FFA is directed into the tissues.
- Thus it helps in the **hydrolysis of Chylomicron and VLDL** to Chylomicron remnant and VLDL remnant respectively.

Lipoprotein Lipase in Heart During Starvation

- Heart lipoprotein lipase has a low K_m for triacylglycerol, about one-tenth of that for the enzyme in adipose tissue.
- This enables the delivery of fatty acids from triacylglycerol to be **redirected from adipose tissue to the heart in the starved state** when the plasma triacylglycerol decreases.
- **Lipoprotein Lipase in Lactating Mammary Gland**
- A similar redirection to the mammary gland occurs during lactation, Allowing uptake of lipoprotein triacylglycerol fatty acid for **milkfat** synthesis.

Lipoprotein Lipase and Insulin

- In adipose tissue, **insulin enhances lipoprotein lipase synthesis in adipocytes** and its translocation to the luminal surface of the capillary endothelium.
- Deficiency of Lipoprotein lipase lead to Type I Hyperlipoproteinemia.

Hepatic Lipase

- Is bound to the sinusoidal surface of liver cells.
- Is also released by heparin.
- This enzyme, however, does not react readily with chylomicrons or VLDL.
- But is involved in chylomicron remnant and HDL metabolism.

Hormone Sensitive Lipase

- The activity of this enzyme is under the control of hormones, hence the name.
- Present in the **adipose tissue**.
- Involved in the metabolism of triacylglycerol stored in the adipose tissue.
- Hence mobilization of fatty acids from adipose tissue during starvation and diabetes mellitus.

43. All of the following statements about apoproteins are true Except: (AI 2008)

a. Apoprotein A-I activates LCAT
b. Apoprotein C-I activates lipoprotein lipase
c. Apoprotein C-II inhibits lipoprotein lipase
d. Apoprotein C-II activates lipoprotein lipase

Ans. c. Apoprotein C-II inhibits lipoprotein lipase

(Ref: Harper 30/e p255)

Apoliporoteins and its function

Apolipoprotein	Function
Apo A-I	Activates Lecithin Cholesterol Acyl Transferase (LCAT)
Apo A-II	Inhibits Lipoprotein Lipase
Apo A-V	Promote lipoprotein lipase mediated Triacyl Glycerol lipolysis.
Apo B-100	Assembly of VLDL in the liver. Act as ligand for the LDL receptor and LDL receptor related protein (LRP-1) for uptake of LDL
Apo B-48	Assembly of Chylomicron in the intestine.
Apo C-I	Inhibit Cholesterol Ester Transfer Protein(CETP)
Apo C-II	Activates lipoprotein Lipase
Apo C-III	Inhibit Lipoprotein Lipase
Apo E	Act as ligand for LDL receptor for uptake of Chylomicron remnant and VLDL remnant (IDL)

44. Which of the following types of Hypertriglyceridemia is associated with an increase in chylomicron and VLDL remnants? (AI 2007)

a. Type I
b. Type IIa
c. Type III
d. TYPE IV

Ans. c. Type III *(Ref: Harrison 18/e Chapter 356 Table 356-4)*

45. The human plasma lipoprotein containing the highest percentage of triacylglycerol by weight is: (AI 2006)

a. VLDL
b. Chylomicrons
c. HDL
d. LDL

Ans. b. Chylomicrons *(Ref: Harper 30/e 254 Table 25-1)*

- Lipoprotein with highest TAG content is Chylomicron
- Lipoprotein with highest Cholesterol and Cholesterol ester content is LDL.

46. Cholesterol from dietary sources is transported to the peripheral tissue by: (Kerala 2012)

a. Chylomicron
b. VLDL
c. LDL
d. HDL

Ans. c. LDL *(Ref: Harper 30/e p272)*

Transport of Cholesterol between tissues

- Dietary cholesterol is incorporated into Chylomicrons.
- 95% of Chylomicron cholesterol is delivered to liver in Chylomicron remnant.
- The cholesterol is secreted from liver in VLDL.
- Most of the cholesterol secreted in VLDL is retained in IDL and ultimately LDL.
- *LDL* cholesterol is taken up by liver and extrahepatic tissues *(peripheral tissues)*.

47. LDL level in NonDiabetics should be below what value in mg/dl? (NBE Pattern Q)

a. 100
b. 75
c. 50
d. 130

Ans. a. 100 mg/dl

(Ref: Tietz: Fundamentals of Clinical Chemistry 6/e p419)

LDL Goals for different risk categories of atherosclerosis	
Risk category	LDL Goal mmol/L(mg/dl)
Very high	< 1.8 (< 70)
High	< 2.6 (< 100)
Moderately high	< 2.6 (< 100)
Moderate	< 3.4 (< 130)
Lower	< 4.1 (< 160)

48. Action of lipoprotein lipase is: (NBE Pattern Q)

a. To form remnant lipoprotein
b. Promote Lipolysis in Adipose tissue
c. To form mature Chylomicron
d. To form HDL

Ans. a. To form remnant lipoprotein

(Ref: Harper 30/e p257)

The Action of Lipoprotein Lipase Forms Remnant Lipoproteins

Reaction with lipoprotein lipase results in the loss of 70–90% of the triacylglycerol of chylomicrons and in the loss of apo C (which returns to HDL) but not apo E, which is retained. The resulting *chylomicron remnant* is about half the diameter of the parent chylomicron and is relatively enriched in cholesterol and cholesteryl esters because of the loss of triacylglycerol. Similar changes occur to VLDL, with the formation of *VLDL remnants* (also called *intermediate-density lipoprotein (IDL)*

49. In Coronary artery disease the cholesterol level (mg/dl) recommended is: (NBE Pattern Q)

a. Below 200
b. < 250
c. < 220
d. < 280

Ans. a. Below 200

(Ref: Tietz: Fundamentals of Clinical Chemistry 6/e p419)

Biochemical parameter	Values mg/dL	Risk
LDL- Cholesterol	< 100	Optimum
	100–129	Near or above Optimum
	130–159	Borderline high
	160–189	High
	> 190	Very High
Total - Cholesterol	< 200	Desirable
	200–239	Borderline High
	> 240	High
HDL-Cholesterol	< 40	High
	> 60	***Low***

50. Lipoprotein x is an indicator of: (NBE Pattern Q)

a. Atherosclerosis
b. Cholestatsis
c. Hepatitis
d. Myocardial Infarction

Ans. b. Cholestasis

In cholestasis, free cholesterol, coupled with phospholipids, is secreted into the plasma as a constituent of a lamellar particle called LP-X. The particles can deposit in skinfolds, producing lesions resembling those seen in patients with FDBL (xanthomata strata palmaris). Planar and eruptive xanthomas can also be seen in patients with cholestasis.

51. Which is the lipoprotein with lowest density?

a. HDL
b. LDL
c. VLDL
d. Lp a

Ans. c. VLDL *(Ref: Harper 30/e p214 Table 25.1)*

Composition of different Lipoproteins

				Composition			
Lipoprotein	Source	Diameter (nm)	Density (g/mL)	Protein (%)	Lipid (%)	Main lipid components	Apolipoproteins
Chylomicrons	Intestine	90–1000	< 0.95	1–2	98–99	Triacylglycerol	A-I, A-II, A-IV, B-48, C-I, C- II, C-III, E
Chylomicron remnants	Chylomicrons	45–150	< 1.006	6–8	92–94	Triacylglycerol, phospholipids, cholesterol	B-48, E
VLDL	Liver (intestine)	30–90	0.95–1.006	7–10	90–93	Triacylglycerol	B-100, C-I, C- II, C-III
IDL	VLDL	25–35	1.006–1.019	11	89	Triacylglycerol, cholesterol	B-100, E
LDL	VLDL	20–25	1.019–1.063	21	79	Cholesterol	B-100
HDL	Liver, intestine, VLDL, chylomicrons			Phospho-lipids, cholesterol	A-I, A-II, A-IV, C-I, C-II, C-III, D, 2 E		
HDL		20–25	1.019–1.063	32	68		

52. Which of the following has highest electrophoretic mobility and least lipid content: (PGI June 01)

a. Chylomicrons
b. HDL
c. LDL
d. VLDL
e. IDL

Ans. b. HDL *(Ref: Harper 30/e p254 Table 25-1)*

HDL

- Alpha lipoprotein
- Least Diameter
- Maximum Electrophoretic Mobility
- Maximum Protein Content
- Least lipid content
- Carry Cholesterol from peripheral tissues to liver and other steroidogenic tissues
- This is called Reverse Cholesterol transport
- This makes HDL Cholesterol 'the good cholesterol'
- The major role of HDL is to acts as the repository for apo C and apo E required for the metabolism of VLDL and Chylomicron.

53. Which helps in the transport of chylomicrons from intestine to liver: (AI 2000)

a. Apoprotein B
b. Apoprotein A
c. Apoprotein C
d. Apoprotein E

Ans. d. Apoprotein E *(Ref: Harper 30/e p257)*

- Transport of Chylomicron remnant to liver by Apo E
- Transport of IDL or VLDL remnant to liver by Apo E
- Transport of LDL to liver and Extrahepatic tissues by apo B100.

54. Which of the following is an activator of LCAT: (JIPMER 2002)

a. Apo B 100
b. Apo B 48
c. Apo E
d. Apo A-I

Ans. d. Apo A-I *(Ref: Harper 30/e p255)*

55. Cholesterol present in LDL: (AIIMS May 03)

a. Represents primarily cholesterol that is being removed from peripheral cells.
b. Binds to a receptor and cholesterol diffuses across the cell membrane.
c. On accumulation in the cell inhibits replenishment of LDL receptors.
d. When enters a cell, suppresses activity of acyl-CoA: cholesterol acyltransferase ACAT

Ans. c. On accumulation in the cell inhibits replenishment of LDL receptors. *(Ref: Harper 30/e p271)*

- *Option a:* LDL cholesterol is primarily from other lipoproteins where as HDL cholesterol is from peripheral organs.
- *Option b:* Mechanism of uptake of LDL by LDL receptor
  - LDL receptors occur on the cell surface in pits that are coated on the cytosolic side of the cell membrane with a protein called **clathrin.**
  - The LDL receptor spans the membrane, the B-100 binding region being at the exposed amino terminal end.

- After binding, LDL is **taken up intact by endocytosis**.
- The apoprotein and cholesteryl ester are then hydrolyzed in the lysosomes, and cholesterol is translocated into the cell.
- The receptors are recycled to the cell surface.

- *Option b:* LDL is taken up intact, and not cholesterol alone diffuses across the membrane.

Cholesterol balance in tissues

This influx of cholesterol inhibits the transcription of the genes encoding

- HMG-CoA synthase, HMG-CoA reductase, and other enzymes involved in cholesterol synthesis
- The LDL receptor itself, via the SREBP pathway

Thus coordinately suppresses cholesterol synthesis and uptake.

- In addition, ACAT activity is stimulated, promoting cholesterol esterification.

Recent advance

In addition, recent research has shown that the protein **proprotein convertase subtilisin/kexin type 9 (PCSK9)** regulates the recycling of the receptor to the cell surface by targeting it for degradation. By these mechanisms, Cholesterol balance is maintained within normal limits in the cell.

56. A person on a fat free carbohydrate rich diet continues to grow obese. Which of the following lipoproteins is likely to be elevated in his blood? (AI 2004)

a. Chylomicrons
b. VLDL
c. LDL
d. HDL

Ans. b. VLDL

Excess Carbohydrates are converted to Acetyl CoA, which is converted to Fatty acid, esterified to produce Endogenous TGs. VLDL carry the endogenoud TGs. So VLDL level rises.

57. Which of the following is false about heparin? (JIPMER May 2014)

a. Releases lipoprotein lipase
b. Releases hormone sensitive lipase
c. It is an anticoagulant
d. It is a Glycosaminoglycan

Ans. b. Releases hormone sensitive lipase

- **Heparin is an anticoagulant and it is a Glycosamino Glycan.**
- **It releases Lipoprotein lipase not hormone sensitive lipase.**

58. Lipoprotein a resembles: (JIPMER 2014)

a. Plasminogen
b. Plasmin
c. Thrombin
d. Prothrombin

Ans. a. Plasminogen *(Ref: Chatterjea and Shinde 8/e p448)*

Lp(a)

- Almost silmilar to LDL.
- Apo (a) is attached to apo B100 by disulphide bond.
- Major site of clearance of Lp(a) is liver.
- Strongly associated with Atherosclerosis and Myocardial infarction.
- Significant homology with Plasminogen^{Q}.
- It interferes with activation of Plasminogen to plasmin.
- Hence fibrin clot is no lysed.
- Susceptible to Intravascular thrombosis.

Dysbetalipoproteinemia

59. Which of the following is increased in lipoprotein lipase deficiency: (AIIMS Nov 2000)

a. VLDL
b. LDL
c. HDL
d. Chylomicrons

Ans. a. VLDL, **d.** Chylomicron

Phenotype	I	IIa	IIb	III	IV	V
Lipoprotein, elevated	**Chylo-microns (pre-domi-nant)** VLDL	LDL	LDL and VLDL	Chylo-micron and VLDL rem-nants	VLDL	Chy-lomi-crons and VLDL
Triglycerides	↑↑↑	N	↑	↑↑	↑↑	↑↑↑
Cholesterol (total)	↑	↑↑↑	↑↑	↑↑	N/↑	↑↑
LDL-choles-terol	↓	↑↑↑	↑↑	↓	↓	↓
HDL-choles-terol	↓↓↓	N/↓	↓	N	↓↓	↓↓↓

60. Familial hypercholesterolemia is: (PGI Dec 98)

a. Deficient LDL receptors
b. Deficient HDL receptors

c. HMG CoA reductase deficiency
d. Deficient VLDL receptors

Ans. a. Deficient LDL receptors

Frederickson type	Nomenclature	Molecular defect
Type I	Familial Chylomicronemia Syndrome (FCS)	Lipoprotein Lipase or Apo CII
Type IIa	Familial	LDL receptor
	Hypercholesterolemia (FH)	
	Familial Defective Apo B (FDB) Autosomal Dominant Hypercholesterolemia Type II (ADH Type II)	Apo B100
	Autosomal Dominant Hypercholesterolemia Type III (ADH Type III)	PCS K9
	Autosomal Recessive Hypercholesterolemia (ARH)	LDL Receptor Adapter Protein (LDLRAP)
	Sitosterolemia	ABCG5 or ABCH8
Type II b	Familial Combined Hyperlipidemia (FCHL)	-----
Type III	Familial Dysbeta lipoproteinemia (FDBL)	Apo E
Type IV	Familial Hypertriglyceridemia (FHTG)	Apo A -V
Type V	Familial Hypertriglyceridemia (FHTG)	Apo A –V and GPIHBP 1

61. Hypertriglyceridemia not seen in: **(CMC Vellore 2014)**

a. Hypothyroidism
b. Type 2 Diabetes Mellitus
c. Cushing's Syndrome
d. Hepatitis

Ans. a. Hypothyroidism *(Ref: Harrison 19/e Table 356-5)*

Conditions associated with increased LDL (Increased Cholesterol)

- Hypothyroidism
- Nephrotic syndrome
- Cholestasis
- Acute intermittent porphyria
- Anorexia nervosa
- Hepatoma
- Drugs: thiazides, cyclosporin, tegretol

Conditions associated with decreased LDL

- Severe liver disease
- Malabsorption
- Malnutrition
- Gaucher's disease
- Hyperthyroidism
- Drugs: niacin toxicity

62. A patient was diagnosed with isolated increase in LDL. His father and brother had the same disease with increased cholesterol. The likely diagnosis is: **(AIIMS May 2009)**

a. Familial type III hyperlipoproteinemia
b. Abetalipoproteinemia
c. Familial LPL deficiency (type1)
d. LDL receptor mutation

Ans. d. LDL receptor mutation

(Ref: Harrison 18/e Table 356.3)

Frederickson's Classification of Hyperlipoproteinemia

Phenotype	Type I	Type II	Type III
Molecular Defect	Lipoprotein Lipase apo CII	LDL receptor defect apo B100 defective	apo E defect
Genetic Nomenclature	Familial Chylomicronemia Syndrome	Familial Hypercholesterolemia Familial Defective apo B	Familial Dysbetalipoproteinemia
Clinical features	Eruptive Xanthoma Pancreatitis Lactescent Plasma No Coronary/ Peripheral Atherosclerosis	Tendon Xanthoma Tuberous Xanthoma No Pancreatitis No Lactescent plasma Coronary atherosclerosis +++ Peripheral Atherosclerosis +	Tuberoeruptive Xanthoma No Pancreatitis Coronary atherosclerosis +++ Peripheral Atherosclerosis +
Lipid elevated	Triacyl Glycerol NB: Cholesterol Normal	Cholesterol NB: Triacyl Glycerol Normal	Cholesterol Triacyl Glycerol
Lipoprotein elevated	Chylomicron	LDL	Chylomicron remnant VLDL remnant

63. Abetalipoproteinemia result in absence of: **(PGI Dec 02)**

a. Chylomicron
b. LDL
c. VLDL
d. HDL
e. TG

Ans. a, b, c, e.

(Ref: Harrison 19/e Chapter Disorders associated with metabolism of Lipoproteins)

Abetalipoproteinemia

- Autosomal recessive disease
- Biochemical Defect
- Loss-of-function mutations in the gene encoding microsomal triglyceride transfer protein (MTP) the gene name MTTP.
- MTP transfers lipids to nascent chylomicrons and VLDLs in the intestine and liver, respectively.
- So Chylomicron^0, VLDL^0 and hence LDL^0 not produced.
- Plasma levels of cholesterol and triglyceride are extremely low in this disorder.
- Chylomicrons, VLDLs, LDLs, and apo B are undetectable in plasma.

64. Absence of this apo lipoprotein is responsible for the genetic disorder, familial type III hyperlipoproteinemia (NBE Pattern Q)

a. Apo B100
b. Apo B48
c. Apo E
d. Apo CII

Ans. c. Apo E

65. Patient with abetalipoproteinemia frequently manifests with delayed blood clotting. This is due to inability to: (JIPMER 2012)

a. Produce chylomicrons
b. Produce VLDL
c. Synthesize clotting factors
d. Synthesize fatty acids

Ans. a. Produce chylomicrons, **b.** Produce VLDL

Fat soluble vitamins are absorbed in Chylomicrons, from liver it is secreted out by VLDL

Vitamin deficiency in Abetalipoproteinemia^Q

- Most clinical manifestations of abetalipoproteinemia result from defects in the absorption and transport of fat-soluble vitamins.
- Vitamin E and retinyl esters are normally transported from enterocytes to the liver by chylomicrons.
- Vitamin E is dependent on VLDL for transport out of the liver and into the circulation.
- As apo B containing lipoproteins are not formed these patients, there is, marked deficiency of Vitamin E, mild to moderate deficiency of Vitamin A and K.
- Bleeding manifestation due to defective absorption of fat and hence fat soluble vitamins.

66. Apolipoprotein of Chylomicron is:

a. Apo B100 **(JIPMER Nov 2015)**
b. Apo B48
c. Apo E
d. Apo AI

Ans. b. Apo B48 *(Ref: Harper 30/e p254)*

- Apo B48 is found exclusively in chylomicron.
- The only one apolipoprotein in LDL is apo B100

67. Both Triglycerides and HDL increased:

a. Smoking **(JIPMER Nov 2015)**
b. Athletes
c. Statin
d. Alcoholism

Ans. d. Alcoholism

(Ref: Harrison 19/e Disorders of lipoprotein metabolism)

- Regular alcohol consumption has a variable effect on plasma lipid levels.
- The most common effect of alcohol is to increase plasma triglyceride levels.
- Alcohol consumption stimulates hepatic secretion of VLDL, possibly by inhibiting the hepatic oxidation of free fatty acids, which then promote hepatic triglyceride synthesis and VLDL secretion.
- Regular alcohol use also raises plasma levels of HDL-C.

68. In prolonged fasting Glycerol formed from Triglyceride. Which of the following statement is true regarding Glycerol: (PGI 2013)

a. Used in synthesis of chylomicron
b. It is directly used by tissues for energy needs
c. It is formed due to increased activity of hormone sensitive lipase
d. Glycerol acts as a substrate for gluconeogenesis
e. It is formed by increased activity of Lipoprotein Lipase

Ans. c. It is formed due to increased activity of hormone sensitive lipase, **d.** Glycerol acts as a substrate for gluconeogenesis

In prolonged fasting Gluconeogenesis sets in and Glycerol liberated from stored TGs in adipose tissue by the action of hormone sensitive lipase.

69. Full form of LCAT: (PGI May 2014)

a. Lecithin Cholesterol Acyl Transferase
b. Lecithin Choline Acyl Transferase
c. Lecithin Cholesterol Alkyl Transferase
d. Lecithin Choline Alcohol Transferase
e. Lecithin CoA Tranferase

Ans. a. Lecithin Cholesterol Acyl Transferase.

4

Section | Molecular Genetics

CHAPTERS

8. Chemistry and Metabolism of Nucleotides
9. Structure and Organization of DNA
10. DNA Replication
11. Transcription
12. Translation
13. Regulation of Gene Expression
14. Molecular Biology Techniques and Recent Advances in Molecular Biology

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8 Chemistry and Metabolism of Nucleotides

Topics Included

- Nucleic Acids
- Components of Nucleotides
- Metabolism of Purines and Disorders
- Metabolism of Pyrimidines and Disorders

NUCLEIC ACIDS

Nucleic acids are polymers of nucleotides joined by 3'–5' phosphodiester bond.

Two Types of Nucleic Acid

- Deoxyribonucleic acid (DNA)
- Ribonucleic acid (RNA).

General Properties of Nucleotides

- Nucleotides **are polyfunctional acids**. Nucleotides bear a **negative** charge at physiological pH.
- Absorbs UV light at a wavelength **260 nm**, at pH 7.0. The conjugated double bond of Purine and Pyrimidine nucleotide is responsible for it.

Ultraviolet Rays are Mutagenic

The mutagenic effect of ultraviolet light is due to its absorption of UV light by nucleotides in DNA that result in chemical modifications.

NUCLEOTIDES

Composition of Nucleotides

The components of nucleotides are:

A nitrogenous base + A pentose Sugar + Phosphate Group

Nitrogenous Base

They are nitrogen containing heterocyclic ring structures.

Two types of Nitrogenous Bases

- Purine
- Pyrimidine.

1. **Purine Bases**Q **are** Adenine and Guanine
   - Adenine-6 Amino Purine
   - Guanine-2-amino, 6-oxopurine.

Adenine and Guanine are present in both DNA and RNA.

Minor Purine Bases are:

- Hypoxanthine-6 oxo purine
- Xanthine-2, 6 dioxopurine
- Uric Acid-2, 6, 8 trioxopurine.

2. **Pyrimidine Bases are** Cytosine, Uracil and Thymine
   - Cytosine-2 oxo 4 amino Pyrimidine
   - Uracil-2, 4 dioxo Pyrimidine
   - Thymine-2, 4 dioxo 5 methyl Pyrimidine.

Modified Nitrogenous bases

- Dihydrouracil
- Pseudouridine
- 5-Methyl Cytosine
- Dimethyl amino adenine
- 7-methyl Guanine.

Remember

- Cytosine is present in both DNA and RNA
- Uracil is present only in the RNA
- Thymine is present only in the DNA.

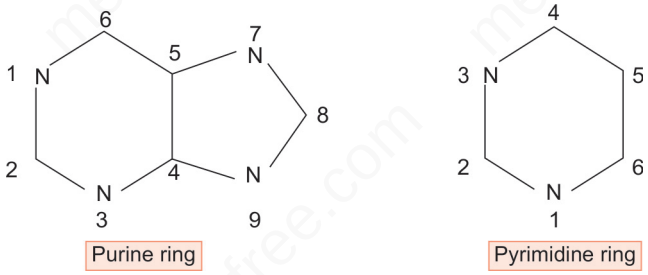


Fig. 8.1: Structure of purine and pyrimidine ring

Methylated Xanthine derivative present in the
Coffee-Caffeine (Trimethylxanthines)
Tea-Theophylline (Dimethylxanthine)
Cocoa-Theobromine (Dimethylxanthine)

Vitamin that contains pyrimidine ring is Thiamine

Pentose Sugar

There are two types of Pentose Sugar.

- D-Ribose Sugar in RNA
- 2′ deoxy D-Ribose Sugar in DNA.

Composition of Nucleoside

- Nitrogenous base + Pentose Sugar
- C1 of ribose or deoxyribose sugar to N1 of Pyrimidine or N^9 of Purine by β N Glycosidic linkage
- So Nucleosides are N Glycosides.

Composition of Nucleotides

- Nucleoside + Phosphoryl groups
- Usually phosphoryl group is attatched to 5′ hydroxyl group of pentose sugar by an ester bond.
- Additional phosphoryl group are attached by an acid anhydride bond to form nucleoside diphosphates and triphosphates.

Ribonucleotides			
Nitrogenous Base	Pentose Sugar	Nucleoside = Nitrogenous Base	Nucleotide = Nucleoside + Phosphate Group
Adenine	Ribose	Adenosine	Adenosine Mono-phosphate (AMP)
Guanine	Ribose	Guanosine	Guanosine Mono-phosphate (GMP)
Cytosine	Ribose	Cytidine	Cytidine Mono-phosphate (CMP)
Uracil	Ribose	Uridine	Uridine Mono-phosphate (UMP)
Hypoxanthine	Ribose	Inosine	Inosine Mono-Phosphate (IMP)
Xanthine	Ribose	Xanthosine	Xanthosine Mono-Phosphate (XMP)
Deoxyribonucleotides			
Adenine	Deoxy-ribose	dAdenosine	dAdenosine Mono-phosphate (dAMP)
Guanine	Deoxy-ribose	dGuanosine	dGuanosine Mono-phosphate (dGMP)
Cytosine	Deoxy-ribose	dCytidine	dCytidine Mono-phosphate (dCMP)
Thymine	Deoxy-ribose	dThymidine	Thymidine Mono-Phosphate

Nucleic Acid

Polymers of nucleotides joined by 3'–5' phosphodiester bond.

They are RNA and DNA.

3′–5′ Phosphodiester bond

3′ hydroxyl group of sugar of first mononucleotide linked to 5′ phosphoryl group of the second mononucleotide by a 3′–5′ phosphodiester bond.

Nucleic Acid Exhibit Polarity

- Nucleotides are linked by 3'–5' phosphodiester bond, there is a free phosphoryl group at 5' of the first nucleotide and free hydroxyl group at the 3' end of last nucleotide.
- Hence, nucleic acid exhibits polarity.
- The base sequence is usually written from 5' end to 3′ end (Fig. 8.2).

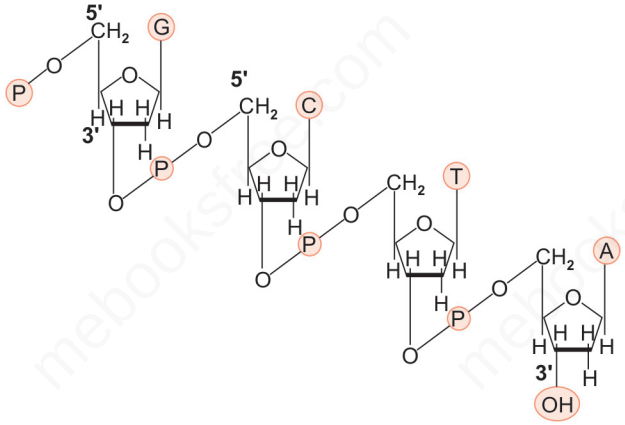


Fig. 8.2: Nucleic acid

Remember
Most abundant free nucleotide in mammalian cell is ATP.

Functions of Nucleotides and Nucleotide Derivative

- They are building blocks of nucleic acid.
- Adenosine Tri-Phosphate (ATP) is the principal biological transducer of free energy.
- cAMP and cGMP are second messengers in hormonal pathways.
- S Adenosyl Methionine (SAM) is an important methyl donor.
- Adenosine 3' Phosphate -5' phosphosulphate (PAPS) is an important Sulfate donor.
- UDP-Sugar derivative in Glycogen synthesis and oligosaccharides of Glycoproteins.
- UDP-Glucuronic acid in conjugation of bilirubin.

- Many coenzymes incorporate nucleotides in its structure, like NAD^+, NADP, FMN, FAD^+

Nucleotide Derivatives as Coenzymes

Biologically active forms of Niacin are NAD^+ and $NADP^+$

NAD^+ is Nicotinamide Adenine Dinucleotide.

- It consists of two nucleotides
- First nucleotide with nicotinamide as base and second nucleotide with adenine as base
- Nicotinamide-D Ribose-P-P-D Ribose-Adenine

$NADP^+$ is Nicotinamide Dinucleotide Phosphate

- To the Adenine of NAD+ there is Phosphoric group

Biologically active form of Riboflavin are FAD^+ and FMN. FMN is Flavin Mononucleotide (FMN).

It consists of Flavin-Ribityl-P, where ribityl is an alcohol.

FAD^+ is Flavin adenine nucleotide

It consists of Flavin-Ribityl-P-P-Ribose-Adenine.

Comparison of DNA and RNA

DNA	RNA
Mostly seen in the Nucleus	Mostly seen in the Cytoplasm
Pyrimidine bases are Thymine and Cytosine	Pyrimidine bases are Uracil and Cytosine
Sugar is deoxyribose	Sugar is Ribose
Pyrimidines = Purines	Pyrimidines not equal to Purines
Not destroyed by Alkali	Destroyed by Alkali
Usually Double Stranded	Single Stranded

Pseudouridine in tRNA

- Seen in the Ribothymidine Pseudouridine Cytidine (TψC) arm of tRNA.
- Formed by modification of UMP on preformed tRNA.
- D-Ribose of pseudouridine linked to C-5 of Uracil, by a carbon to carbon bond rather than by β N Glycosidic bond.
- Pseudouridine is excreted unchanged in urine.

Thymidine in tRNA

- UMP in preformed tRNA is methylated by S-adenosyl Methionine (SAM) to form TMP.
- tRNA is the RNA with thymidine.
- Ribithymidine is also seen in Pseudouridine Cytidine (TψC) arm of tRNA.

PURINE METABOLISM

Metabolic Pathways of Purine

- De novo synthesis of Purine Nucleotides
- Salvage pathways of Purine Nucleotides
- Degradation of Purine Nucleotides.

De Novo Synthesis of Purine Nucleotides

- Site of synthesis—Most of the tissues but majority in the liver
- Organelle—Cytoplasm.

Sites where de novo synthesis do not take place:

- Brain
- Erythrocytes
- Polymorphonuclear leukocytes
- Bone marrow.

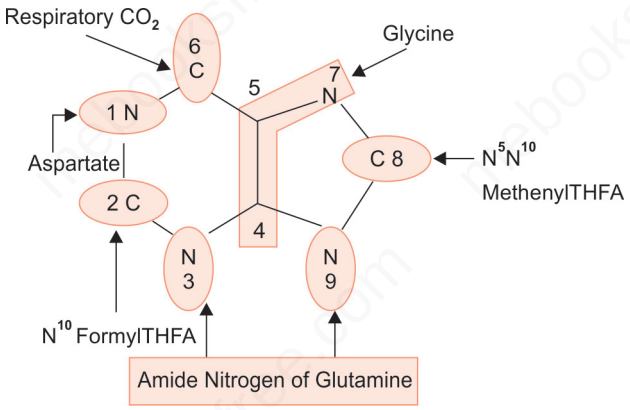


Fig. 8.3: Source of purine ring

Sources of Purine Ring

- C 4, C 5, N7 by Glycine
- N3, N 9 by Amide Nitrogen of Glutamine
- N 1 by Aspartate
- C2 by N^{10} Formyl THFA
- C8 by N^5 N^{10} Methenyl THFA
- C6 by Respiratory CO_2

Key Points of De Novo Purine Biosynthesis

- Rate limiting Step in Purine Synthesis—PRPP Glutamyl Amidotransferase
- Purine ring is built up on a ribose-5-phosphate. Hence, nucleotides are the products of the de novo synthesis
- First Nucleotide formed in Purine Synthesis-Inosine Mono Phosphate (IMP)
- From IMP Adenosine Monophosphate (AMP) and Guanosine monophosphate (GMP) is formed.

Conversion of IMP to AMP and GMP

- Amino group of AMP is contributed by Aspartic Acid
- Amino group of GMP is contributed by Glutamine.

Mnemonic

A for A (Aspartic Acid for AMP)

G for G (Glutamine for GMP)

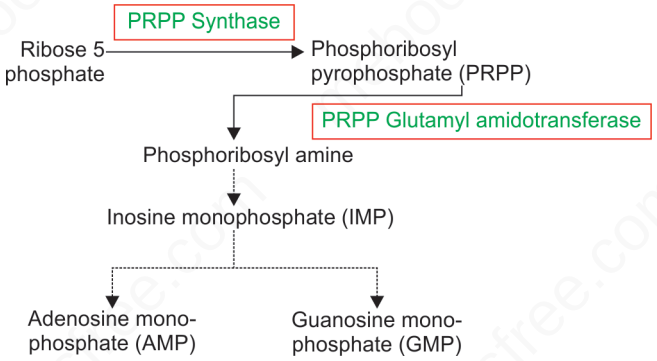


Fig. 8.4: Overview of de novo purine synthesis

Regulation of De Novo Purine Synthesis

- The regulatory enzymes of Purine Synthesis are PRPP Synthase and PRPP Glutamyl Amidotransferase
- The rate limiting step of de novo Purine Synthesis is PRPP Glutamyl amidotransferase
- The overall determinant of de novo purine synthesis is level of PRPP
- This activates PRPP Synthase
- The rate of PRPP Synthesis depends on the availability of ribose 5 Phosphate
- AMP, ADP, GMP and GDP the end products of de novo Purine synthesis feedback regulate PRPP Synthase and PRPP Glutamyl Amidotransferase.

SALVAGE PATHWAY OF PURINE NUCLEOTIDES

Recycling of degraded purines and purine nucleosides to their corresponding mononucleotides.

- Less energy consuming
- Effective recycling of degraded nucleotides
- Important in organs where de novo synthesis do not take place.

Salvage pathway reactions are:

1. Phosphoribosylation of Purine bases (Pu) by PRPP.
2. Phosphorylation of Purine Nucleosides (PuR) by ATP.

Salvage Pathway Reaction of Purines

- Phosphorylation of Purine (Pu) by PRPP

  Adenine Phosphoribosyl Transferase (APRTase)

  – Adenine + PRPP ----------> Adenosine Monophosphate + PPi

  Hypoxanthine Guanine Phosphoribosyl Transferase (HGPRTase)

  – Hypoxanthine + PRPP ----------> Inosine Monophosphate + PPi

  – Guanine + PRPP ----------> Guanosine Monophosphate + PPi

- Phosphorylation of Purine Nucleoside

  Adenosine Kinase

  – Adenosine + ATP ----------> Adenosine Monophosphate + ADP

CATABOLISM OF PURINE NUCLEOTIDES

- Adenine nucleotides catabolism—Liver, Heart muscle, Skeletal muscle, GIT mucosa
- Guanine nucleotide catabolism—Liver, spleen, Kidneys Pancreas, GIT mucosa
- The end product of Purine catabolism is in humans Uric Acid[Q]
- The end product of purine catabolism in mammals other than higher primates is Allantoin
- Uricase enzyme convert uric acid to water soluble, Allantoin.

Steps of Catabolism of Purine Nucleotides

Adenosine Deaminase

- Adenosine converted to inosine by Adenosine Deaminase.

Purine Nucleoside Phosphorylase

- Inosine is converted to Hypoxanthine
- Guanosine is converted to Guanine.

Xanthine Oxidase

- Convert Hypoxanthine and Guanine to Xanthine.
- Same enzyme catalyse conversion of Xanthine to Uric acid.

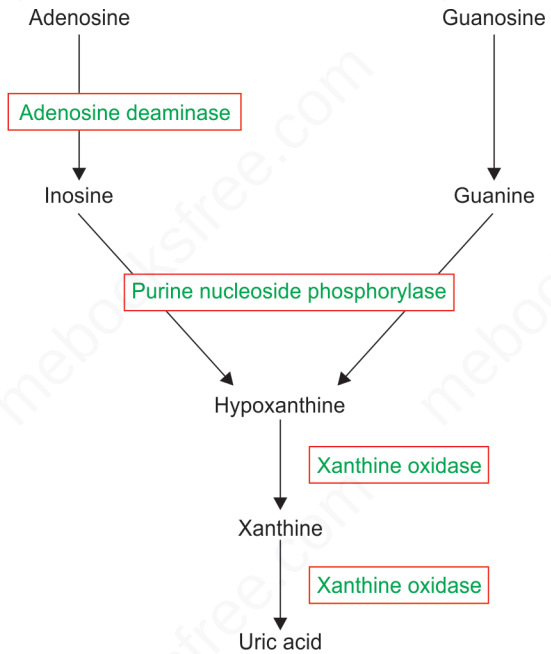


Fig. 8.5: Catabolism of purine nucleosides

CLINICAL CORRELATIONS—PURINE METABOLISM

Lesch–Nyhan Syndrome[Q]

X-linked Recessive Disorder[Q]

Biochemical Defect

- Complete deficiency of HGPRTase
- Purine accumulates
- Purines degraded to uric acid
- Uric acid level increases

Clinical Features

- Hyperuricemia, Intellectual Disability
- Dystonic Movement, Choreoathetosis, Dysarthric Speech
- Compulsive self-mutilation[Q]
- Megaloblastic anemia also can occur in the Lesch–Nyhan syndrome, in which regeneration of purine nucleotides is blocked.

Diagnosis

- Hyperuricemia
- HGPRTase enzyme activity in the erythrocytes is deficient.

Treatment

- Allopurinol
- Alkalanization of urine
- High fluid intake.

Kelley–Seegmiller Syndrome

- Partial deficiency of HGPRTase with >1.5–2% enzyme activity.
- Associated with hyperuricemia and variable neurologic dysfunction.

Adenine Phosphoribosyltransferase (APRTase) Deficiency

Autosomal recessive.

Biochemical Defect

- Deficiency APRTase
- Adenine accumulates
- Adenine oxidized by xanthine dehydrogenase to **2, 8-dihydroxyadenine**, which is extremely insoluble.

Clinical manifestations

- Urinary calculus formation with crystalluria
- The presence of brownish spots on the infant's diaper or of yellow-brown crystals in the urine is suggestive of the diagnosis.

Laboratory findings

- Urinary levels of adenine, 8-hydroxyadenine, and 2, 8-dihydroxyadenine are elevated
- Plasma uric acid is normal.

Treatment includes

- High fluid intake
- Dietary purine restriction
- Allopurinol, which inhibits the conversion of adenine to 2,8-dihydroxyadenine
- *Alkalinization of the urine is to be avoided*, because, unlike that of uric acid, the solubility of 2,8-dihydroxyadenine does not increase up to a pH of 9
- Shock-wave lithotripsy has been reported to be successful.

Gout

Group of disorders presented with

1. Hyperuricemia
2. Uric Acid Nephrolithiasis
3. Acute Inflammatory Arthritis.

Two Types of Gout

- **Primary Gout**—Due to defect in the enzymes that lead to overproduction of purine nucleotides
  - Superactivity PRPP Synthetase (X linked Disorder)
  - Superactivity PRPP Amidotransferase
  - Deficiency of HGPRTase (Lesch–Nyhan syndrome)
  - Glucose 6 Phosphatase Deficiency (Type I Glycogen Storage Disorder).
- **Secondary Gout**

Increased production of uric acid

Leukemia, Lymphoma.

Decreased excretion rate

Renal failure, Thiazide diuretics, Lactic acidosis.

Clinical Features

- **Acute Gouty Arthritis**

  Typically in the metatarsophalangeal joint of the big toe.
- **Chronic Cases**

  Tophi[Q] deposits of monosodium urate crystals in the subcutaneous tissue.

Diagnosis

Aspiration and examination of synovial fluid

- Negatively birefringent[Q] needle shaped **monosodium urate crystals** using polarized light microscopy.

Treatment

- Colchicine, an anti-inflammatory agent

- Uricosuric agents, such as probenecid or sulfinpyrazone
- Allopurinol.

Allopurinol is converted in the body to oxypurinol (alloxanthine), which inhibits xanthine oxidase.

Severe Combined Immunodeficiency (SCID)

Adenosine Deaminase (ADA) defect is one of the causes.

- Both B cells and T cells are affected
- First disorder to be treated by Gene Therapy[Q]
- Enzyme Replacement Therapy with Polyethylene glycol modified bovine adenosine deaminase (PEG-ADA).

Father of Gene Therapy ---- French Anderson

Immunodeficiency in SCID is due to

- Deficiency of ADA
- Adenosine accumulate
- Adenosine converted to its ribonucleotides and deoxyribonucleotides (dATP)
- dATP inhibit ribonucleotidereductase
- Decreases production of all deoxyribose containing nucleotides
- Hence, DNA synthesis decreased.
- Decrease in both T-cells and B-cells, Hence, immunodeficiency.

Purine Nucleoside Phosphorylase Defect

- Severe deficiency of T cells but apparently normal B-cell function is[Q].
- **This is an enzyme in Purine Catabolism.**

Comparison of Immunodeficiency in SCID and Purine Phosphorylase deficiency

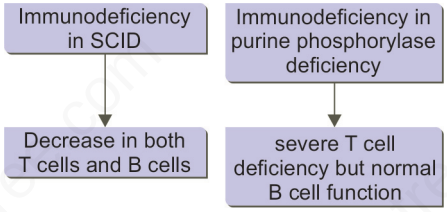


Fig. 8.6: Immunodeficiency in SCID and purine phosphorylase deficiency

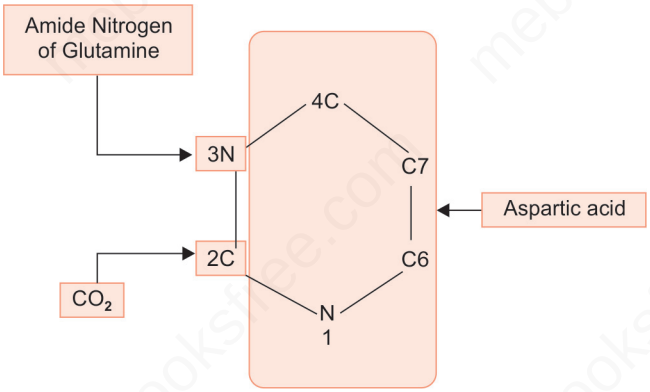
Xanthine Oxidase Deficiency

- Genetic defect in Xanthine Oxidase enzyme
- Associated with Hypouricemia
- Xanthine crystals in urine
- Xanthine lithiasis.

PYRIMIDINE BIOSYNTHESIS

- Site mainly liver
- Organelle Cytoplasm and Mitochondria.

Sources of Pyrimidine Ring



Sources of Pyrimidine Ring

- N3 by Amide Nitrogen of Glutamine
- C2 by CO_2
- C4, C5, C6, N1 by Aspartate

Steps of Pyrimidine Biosynthesis

This pathway is similar to urea cycle.

Enzymes of Pyrimidine Synthesis

- ***Catalyzed by multifunctional enzymes (Single polypeptide with more than one enzyme activity).***

i. **C**PS II, **A**spartate Transcarbamoylase, **D**ihydro Orotase (CAD) in the Cytoplasm

ii. Dihydroorotate Dehydrogenase
   - Only mitochondrial step in Pyrimidine Synthesis.

iii. Orotate Phosphoribosyltransferase and OMP Decarboxylase (Orotidylic acid Decarboxylase)
   - Together called UMP Synthase
   - Seen in the Cytoplasm.

Important Points of Pyrimidine Synthesis

In de novo Purine Synthesis amphibolic intermediates are added on Ribose 5 Phosphate. Hence, Purine Nucleotide is the end product. But in Pyrimidine Synthesis, amphibolic intermediates are not added on a ribose 5 phosphate. Hence, Pyrimidine base is synthesized rather than Pyrimidine nucleotide.

On Pyrimidine base Ribose and Phosphate is added by Orotate Phosphoribosyl Transferase (OPRTase), OMP is formed.

Synthesis of Uridine Nucleotides

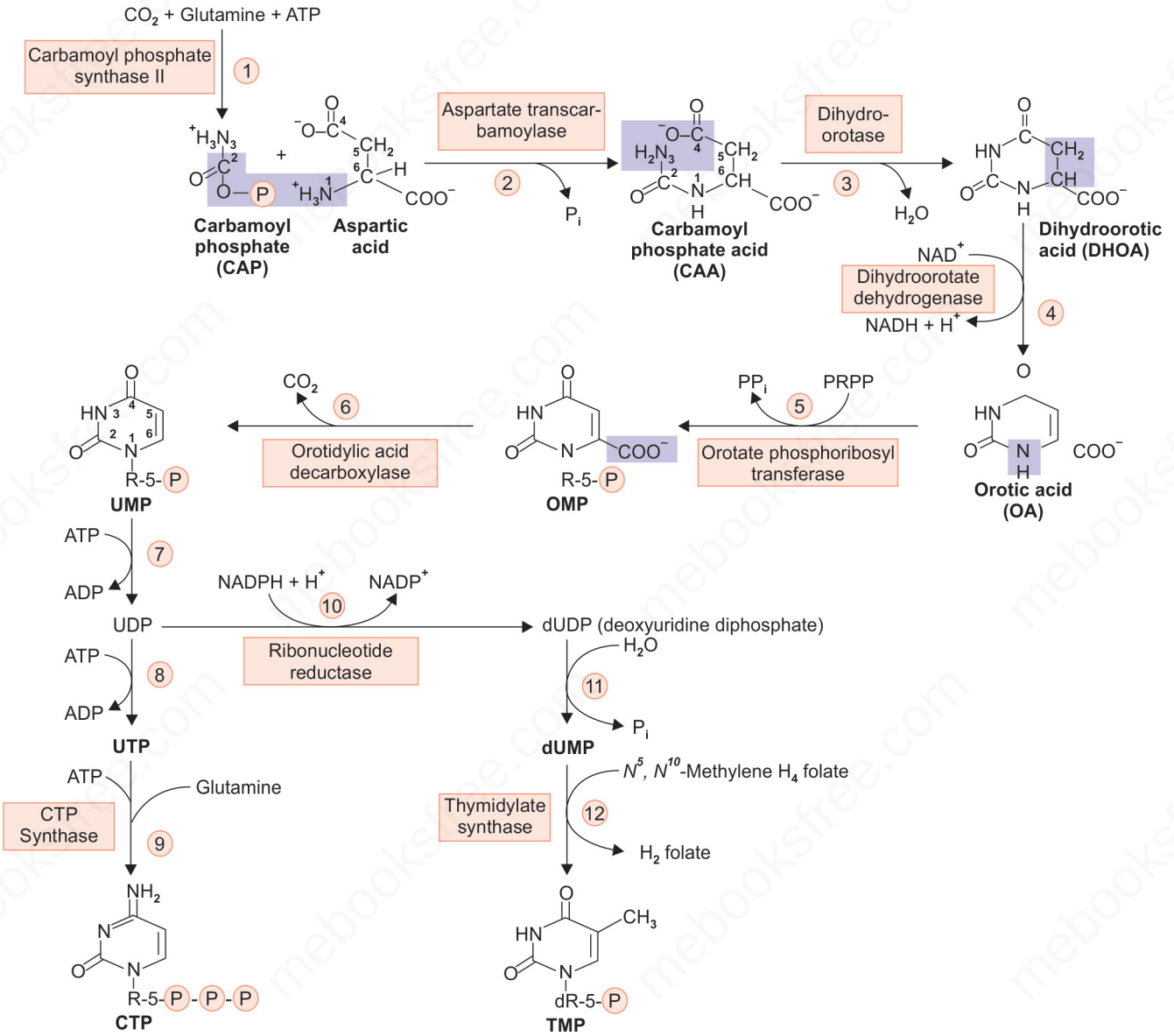
- OMP is decarboxylated to UMP, the first Pyrimidine nucleotide by OMP Decarboxylase.

Synthesis of Cytidine Nucleotides

- UTP converted to CTP by CTP synthase
- Amino group is donated by Glutamine.

Synthesis of Thymine Nucleotides

- First UDP is converted to dUDP by ribonucleotide reductase (explained later)
- dUDP is converted to dUMP
- dUridine Monophosphate[Q] (dUMP) to Thymidine Monophosphate (TMP) by Thymidylate Synthetase
- N^5 N^{10} Methylene THFA donates the methyl group for thymidine Monophosphate (TMP)
- The only reaction in pyrimidine Synthesis where a Tetrahydrofolate derivative is needed.



Two Anticancer Drug Inhibit the Synthesis of TMP

Methotrexate

- Inhibit Dihydrofolate Reductase
- Dihydrofolate Reductase convert Dihydrofolate to Tetrahydrofolate
- Hence, TMP Synthesis is affected.

5 Fluorouracil

- Competitively inhibit Thymidylate Synthase.

Regulation of Pyrimidine Synthesis

- **C**PS II, **A**spartate Transcarbamoylase, **D**ihydro orotase (CAD) is the primary focus of regulation of Pyrimidine Synthesis
- Expression of CAD gene is controlled at genetic level
- CPS II is activated by PRPP and is feedback inhibited by UTP.

Differences between CPS-I and CPS-II

	CPS-I	CPS-II
Cellular location	Mitochondria	Cytosol
Pathway involved	Urea cycle	Pyrimidine synthesis
Source of nitrogen	Ammonia	Amide nitrogen of glutamine
Allosteric regulators	Activator-N-Acetyl Glutamate No inhibitors	Activator-PRPP Inhibitor-UTP

Conversion Ribonucleotides to Deoxyribonucleotides

- Forms deoxyribonucleoside diphosphates (dNDPs) from ribonucleoside diphosphate[Q]
- Ribonucleotide reductase complex catalyses the reaction
- Reduction requires thioredoxin, [Q]thiore-doxin re-ductase, and NADPH.

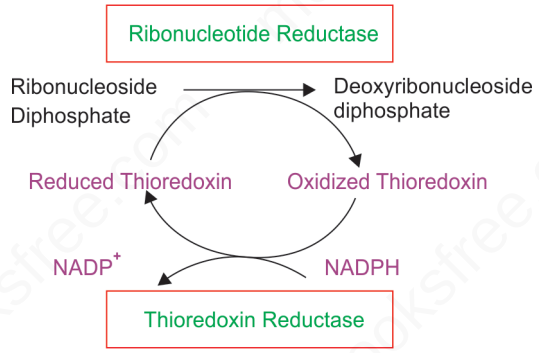


Fig. 8.7: Formation of deoxyribonucleosides

Catabolism of Pyrimidines

- ***Cytosine and Uracil to Beta Alanine CO_2, NH_3***
- ***Thymine to β–aminoisobutyrate CO_2, NH_3***
  - Unlike the end products of purine catabolism, the end products of pyrimidine catabolism are the highly water soluble.
  - Hence, pyrimidine overproduction results in few clinical signs or symptoms.

Clinical Correlation: Pyrimidine Metabolism

Orotic Acidurias

- Rare autosomal recessive condition
- The most common metabolic error in the de novo synthesis of pyrimidines.

Biochemical defect

- **Type I Orotic Aciduria**
  Deficiency of both orotate phosphoribosyltransferase and OMP decarboxylase (together called UMP Synthase).
- **Type II Orotic Aciduria**
  Deficiency only of orotidylate decarboxylase

Clinical Features of Orotic Acidurias

Manifest in the first year of life and is characterized by growth failure, developmental retardation, megaloblastic anemia, and increased urinary excretion of orotic acid.

Diagnosis of Orotic Aciduria

- The diagnosis of this disorder is suggested by the presence of severe megaloblastic anemia with normal serum B_{12} and folate levels and no evidence of TC-II deficiency.
- A presumptive diagnosis is made by finding increased urinary orotic acid.
- Confirmation of the diagnosis, however, requires assay of the transferase and decarboxylase enzymes in the patient's erythrocytes.
- The anemia is refractory to vitamin B_{12} or folic acid but responds promptly to administration of uridine.

Reye Syndrome and Orotic Aciduria

- Inability of severely damaged mitochondria to utilize carbamoyl phosphate,
- Exits to the cytosol, where it stimulates pyrimidine nucleotide biosynthesis.

Urea Cycle Disorder and Orotic Aciduria

- Deficiency in liver mitochondrial ornithine transcarbamoylase .
- Excess carbamoyl phosphate in the mitochondria
- Exits to the cytosol, where it stimulates pyrimidine nucleotide biosynthesis.

Drugs may Precipitate Orotic Aciduria

- **Allopurinol** is an alternative substrate for orotate phosphoribosyltransferase competes with orotic acid.
- **6-Azauridine**, following conversion to 6-azauridylate, also competitively inhibits orotidylate decarboxylase enhancing excretion of orotic acid and orotidine.

Antimetabolites used in Cancer Chemotherapy

Compounds with structural similarity to precursors of purines or pyrimidines (Synthetic Nucleotides), or compounds that interfere with purine or pyrimidine synthesis are called antimetabolites.

- Methotrexate inhibits dihydrofolate reductase competitively.
- Hydroxyurea inhibits ribonucleotide reductase
- 6-Mercaptopurine-Purine analog
- 6-Thioguanine-Nucleotide analog
- 6-Azaguanine—Nucleotide analog
- Aza Serine—Glutamine Analog
- Diazenorleucine—Inhibits PRPP Glutamyl Amido-transferase
- Mycophenolic Acid[Q]—Potent Reversible uncompetitive inhibitor of IMP Dehydrogenase.
- 5-Fluorouracil (5FU)[Q] inhibits thymidylatesynthetase
- 6 Aza-cytidine—inhibits thymidylate synthetase
- 6 Aza-uridine—inhibits thymidylate synthetase
- Cytosine Arabinoside—Ribose is replaced by Arabinose.

- Synthetic nucleotide used in the treatment of Gout—Allopurinol.
- Synthetic nucleotide used in the treatment of AIDS—Zidovudine.
- Synthetic nucleotide used in the treatment of Herpetic Keratitis—5-iodo-deoxyuridine.
- Synthetic nucleotide used to suppress immunologic rejection during organ transplantation—Azathioprine.

REVIEW QUESTIONS

CHEMISTRY OF NUCLEOTIDES

1. Nucleoside is made up of: (PGI Nov 2010)

a. Pyrimidine
b. Histone
c. Sugar
d. Purine
e. Phosphate

Ans. a, c, d. Pyrimidine, Sugar, Purine

(Ref: Harper 30/e page 340)

Composition of Nucleoside

- Nitrogenous base + Pentose Sugar
- C1 of ribose or deoxyribose sugarto N^1 of Pyrimidine or N^9 of Purine by β N Glycosidic linkage.

Composition of Nucleotides

- Nucleoside + Phosphoryl groups
- Usually phosphoryl group is attatched to 5′ hydroxyl group of pentose sugar by an ester bond.
- Additional phosphoryl groups are attached by an Acid anhydride bond to form Nucleoside diphosphates and triphosphates

2. The followings correctly arranged: (PGI June 2009)

a. GMP-Guanine monophosphate
b. UMP-Uracil monophosphate
c. TMP-Thymine monophosphate
d. CMP-Cytidine monophosphate
e. AMP-Adenine monophosphate

Ans. d. CMP-Cytidine monophosphate

(Ref: Harper 30/e page 341)

Ribonucleotides			
Nitrogenous Base	Pentose Sugar	Nucleoside	Nucleotide
Adenine	Ribose	Adenosine	Adenosine Mono-phosphate (AMP)
Guanine	Ribose	Guanosine	Guanosine Mono-phosphate (GMP)
Cytosine	Ribose	Cytidine	Cytidine Mono-phosphate (CMP)
Uracil	Ribose	Uridine	Uridine Mono-phosphate (UMP)
Hypoxanthine	Ribose	Inosine	Inosine Mono-Phosphate (IMP)
Xanthine	Ribose	Xanthosine	Xanthosine Mono-Phosphate (XMP)
Deoxyribonucleotides			
Adenine	Deoxy-ribose	dAdenosine	dAdenosine Mono-phosphate (dAMP)
Guanine	Deoxy-ribose	dGuanosine	dGuanosine Mono-phosphate (dGMP)
Cytosine	Deoxy-ribose	dCytidine	dCytidine Mono-phosphate (dCMP)
Thymine	Deoxy-ribose	Thymidine	Thymidine Mono-Phosphate

3. Apart from occurring in nucleic acid, pyrimidines are also found in: (AIIMS Nov 05)

a. Theophylline
b. Theobromine
c. Flavin mononucleotide
d. Thiamine

Ans. d. Thiamine

- Vitamin with pyrimidine ring is Thiamine
- Theophylline and Theobromine has Purine ring
- FMN also contains Purine ring.

4. Which of the following is not a nitrogenous base?

a. Adenine
b. Guanosine
c. Cytosine
d. Thymine

Ans. b. Guanosine *(Ref: Harper 30/e p 341 table 32-1)*

- Guanosine is nucleoside.

5. Which is not found in DNA: (AI 1994)

a. Adenine
b. Thymine
c. Guanine
d. Uracil

Ans. d. Uracil *(Harper 30/e page 360)*

- Uracil is found only in the RNA.

6. At the physiological pH, the DNA molecules are: (AIIMS Nov 02)

a. Positively charged
b. Negatively charged
c. Neutral
d. Amphipathic

Ans. b. Negatively charged

- DNA is negatively charged because of Phosphate group.

Metabolism of Purines and Pyrimidines

7. End product of purine metabolism in non-primate mammals is: (AIIMS May 2008)

a. Uric acid
b. Ammonia
c. Urea
d. Allantoin

Ans. d. Allantoin *(Ref: Harper 30/e page 354)*

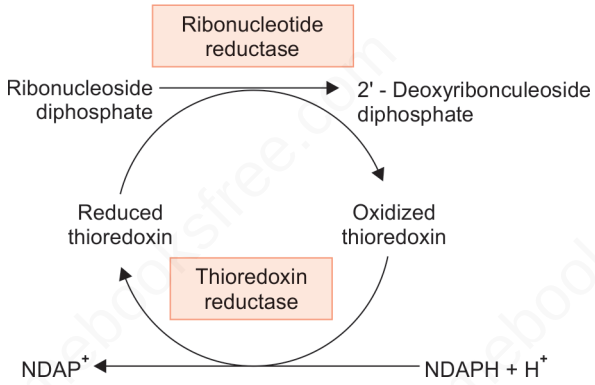
- Humans convert adenosine and guanosine to uric acid
- In mammals other than higher primates, uricase converts uric acid to the water-soluble product allantoin.
- Humans lack uricase, the end product of purine catabolism in humans is uric acid.

8. Deoxyribonucleic acid is formed from:

a. Ribonuclease
b. Ribonucleotide monophosphate
c. Ribonucleotide diphosphate
d. Rubonucleotide triphosphate

Ans. c. Ribonucleotide diphosphate *(Ref: Harper 30/e page 352)*

Reduction of the 2′-hydroxyl of purine and pyrimidine ribonucleotides, catalyzed by the ribonucleotide reductase complex, provides the deoxyribonucleoside diphosphates (dNDPs) needed for both the synthesis and repair of DNA.



9. What is involved in formation of d-TMP from d-UMP? (PGI June 07)

a. N^5, N^{10}-methylene tetrahydrofolate
b. From iminofolate
c. N5 formylfolate
d. Dihydrofolate

Ans. a. d. N^5N^{10} methylene THF and DHF *(Harper 30/e page 353)*

When TMP is formed from dUMP, N^5 N^{10} Methylene THFA is converted to Dihydrofolate

10. Inosinic acid is biological precursor: (Nimhans 97, JIPMER 04)

a. Uracil and thymine
b. Purines and thymine
c. Adenylic acid and guanylic acid
d. Orotic acid and uridylic acid

Ans. c. Adenylic acid and Guanylic acid *(Harper 30/e page 350)*

- AMP and GMP is derived from IMP (Inosinic acid)

11. False regarding gout is: (AI 2001)

a. Due to increased metabolism of pyrimidines
b. Due to increased metabolism of purines
c. Uric acid levels may not be elevated
d. Has a predilection for the great toe

Ans. a. Due to increased metabolism of pyrimidines. Remember Uric acid level may not be elevated always in gout.

12. The enzyme deficient in Lesch-Nyhan syndrome is: (PGI June 99)

a. GTRT
b. Glutaminase

c. Transcarboxylase
d. HGPRTase

Ans. d. HGPRTase *(Harper 30/e page 350)*

13. A 10-year-old child presents with history of rashes self mutilation family history positive which of the following investigations do you think may be suggestive of valuable for diagnosis: (AI 2012)

a. Lead
b. Alkaline phosphatase
c. L D H
d. Uric acid

Ans. d. Uric acid *(Harper 30/e page 350)*

Lesch-Nyhan Syndrome

X-linked Recessive Disorder

Biochemical Defect

- Complete deficiency of HGPRTase
- Purine accumulates
- Purines degraded to uric acid
- Uric acid level increases.

Clinical Features

- Hyperuricemia, Intellectual disability, Dystonic movement, Choreoathetosis, Dysarthric speech.
- Compulsive self-mutilation.

Diagnosis

- Hyperuricemia
- HGPRTase Enzyme activity in the Erythrocytes is deficient.

Treatment

- Allopurinol.

14. A ten-year-old child with aggressive behavior and poor concentration is brought with presenting complaints of joint pain and reduced urinary output. Mother gives history of self-mutilate his finger. Which of the following enzymes is likely to be deficient in this child: (AI 2009)

a. HGPRTase
b. Adenosine deaminase
c. APRTase
d. Acid maltase

Ans. a. HGPRTase *(Harper 30/e page 350)*

15. A patient with increased Hypoxanthine and Xanthine in blood with hypouricemia which enzyme is deficient?

a. HGPRTase
b. Xanthine oxidase
c. Adenosine deaminase
d. APRtase

Ans. b. Xanthine oxidase *(Harper 30/e page 355)*

Hypouricemia

- Hypouricemia and increased excretion of hypoxanthine and xanthine are associated with xanthine oxidase deficiency.

Lesch–Nyhan Syndrome

- The Lesch–Nyhan syndrome, an overproduction hyperuricemia characterized by frequent episodes of uric acid lithiasis and a bizarre syndrome of self-mutilation, reflects a defect in **hypoxanthine-guanine phosphoribosyltransferase**, an enzyme of purine salvage.

Adenosine Deaminase Deficiency

- Adenosine deaminase deficiency is associated with an immunodeficiency disease in which both thymus-derived lymphocytes (T cells) and bone marrow-derived lymphocytes (B cells) are sparse and dysfunctional. Patients suffer from severe immunodeficiency.

Purine Nucleoside Phosphorylase Deficiency

- Purine nucleoside phosphorylase deficiency is associated with a severe deficiency of T cells but apparently normal B cell function.

16. Choose the incorrect statement. Lesch-Nyhan Syndrome: (Kerala 2015)

a. Affects young boys
b. Presents with gouty arthritis
c. The enzyme defect enhances the reutilization of purine bases
d. Bizarre behavior of self-mutilation

Ans. c. The enzyme defect enhances the reutilization of purine bases *(Harper 30/e page 354)*

Lesch-Nyhan Syndrome is characterized by

- X-linked recessive inheritance
- Over production hyperuricemia
- Frequent episodes of uric acid lithiasis
- Bizarre syndrome of self-mutilation.
- Defective reutilzation of Purine bases because of absence of HGPRTase enzyme.

17. Hyperuricemia is not found in:

a. Cancer
b. Psoriasis
c. Von Gierke's disease
d. Xanthinuria

Ans. d. Xanthinuria *(Harper 30/e page 355)*

9 Structure and Organization of DNA

Topics Included

- Structure of DNA
- Organization of DNA
- Supercoiling of DNA
- Central Dogma of Molecular Biology

STRUCTURE OF DNA

Watson, Crick, and Wilkins to propose a model of a DNA in 1953 based on the X-ray diffraction[Q] photographs of DNA taken by Rosalind Franklin.

Salient Features of Watson–Crick Model of DNA

1. Right-handed double stranded DNA helix
2. Base Pairing Rule
   - Adenine always pairs with Thymine
   - Guanine pairs with Cytosine.
3. Two strands are antiparallel.
   The polarity of DNA is such that
   - One strand runs in 5′ to 3′ direction
   - Other strand runs in 3′ to 5′ direction.
4. Hydrogen Bonding
   - Adenine pair with Thymine by 2 Hydrogen bonds. (A = T)
   - Guanine pair with Cytosine by 3 Hydrogen bonds (G = C)
5. Grooves of the DNA
   They are two types:
   1. Major groove
   2. Minor groove
      - Grooves often act as sites of DNA–Protein interaction needed for regulation of gene expression.
      - The DNA Protein interaction is via hydrophobic interaction and ionic bond.

Chargaff's Rule—The number of Purines = The number of Pyrimidines

Different Types of DNA

There are 6 types of DNA.

- A, B, C, D, E -are right handed
- Z is left handed

Characteristics	A-DNA	B-DNA	Z-DNA
Number of base pairs per turns	11	10	12
Morphology	Broad and short	Longer and thinner	Elongated and thin
Base pair tilts the axis of helix	20° tilt	Base pair perpendicular to helix	9° tilt
Screw sense	Right handed	Righ handed	Left handed

A-DNA

- X-ray diffraction studies on dehydrated DNA fibers revealed A form, called A-DNA.
- A-DNA is found in conditions of low humidity and high salt concentration.
- Right-handed double helix like B-DNA.

B-DNA

- Physiologically most common form
- Right-handed double helix
- B-DNA is found in conditions of high humidity and low salt concentration
- Highly flexible.

Z–DNA

- Phosphodiester backbone assume a zig-zag form

- Left-handed double helix
- Seen in the 5′ end of chromosomes
- Longer and thinner than B-DNA
- 12 bp per turn
- Particularly seen in sequence of alternating purine and pyrimidine
- Particularly in d(GC)n sequence
- Methylation of Guanine and Cytosine residues stabilizes Z-form
- Sequences that are not strictly alternating purine and pyrimidine also form Z DNA on methylation
- Z-DNA influences gene expression and regulation.

Points to Ponder

- Physiologically most common is B-DNA.Q
- Under low salt and high degree of hydration B-DNA is usually found
- Under high salt concentration and low degree of hydration A-DNA is usually found.
- The distance spanned by one turn of B DNA is 3.4 nm (34 A^0)
- The width of the double helix in B-DNA is 2 nm (20A^0)

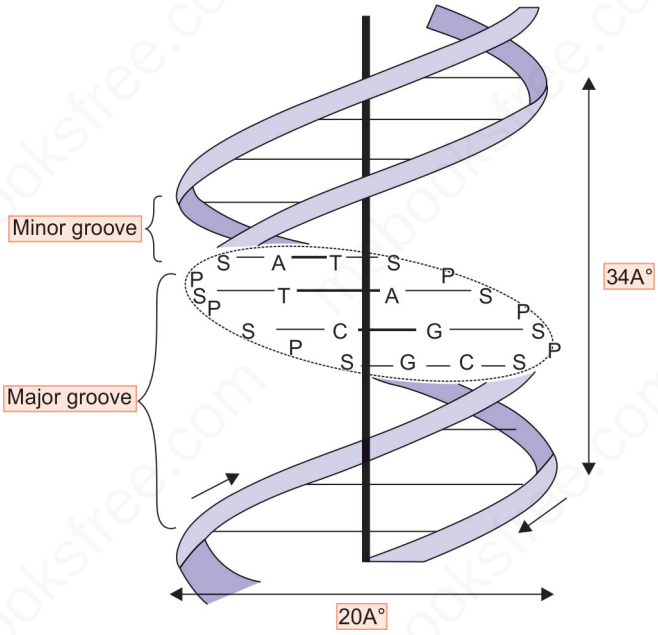


Fig. 9.1: Structure of DNA

NONCANONICAL DNA STRUCTURES

Triple-stranded DNAQ

- Triple-stranded DNA is generated by the hydrogen bonding of a third strand into the major groove of B-DNA
- The third strand forms hydrogen bonds with another surface of the double helix through so-called **Hoogsteenpairs**.Q
- Poly (dA) and Poly (dT) strands combine to form triple stranded DNA.

Four Stranded DNA

- Four Stranded Structure formed in DNA **high in guanine content**
- Ends of eukaryotic chromosomes (telomeres) contain Guanine-rich sequences
- A base pairing scheme for parallel four-stranded DNA, referred to as a **G-quartet DNA**Q
- **Hoogsteen pairs** are seen in four stranded DNA also.

Methods to separate DNA

- DNA can be separated by HPLC, thin layer chromatography (TLC), Paper Chromatography and Gel electrophoresis.

Denaturation of DNA (Melting of DNA)

- The process by which two strands are separated into component strands.

Features of Denaturation

- Breaking of Hydrogen bonds
- Phosphodiester bond is not broken
- Primary structure not altered, only secondary and tertiary structure altered
- Viscosity decreasesQ
- Increase in the optical absorbance of UV light by Purine and Pyrimidine bases, called hyperchromicity.Q

Melting Temperature (Tm)

The strands of a given molecule of DNA separate over a range of temperature. The midpoint is called melting temperature.

Factors Influencing Tm

1. Base Composition.
   - More GC pairs more the Tm.
2. Salt concentration.
   - 10-fold increase of monovalent cation concentration increases the Tm by 16.6°C.
3. FormamideQ destabilize hydrogen bond, hence decreases Tm.

Application of Denaturation of DNA

- Measurement of increased optical absorbance at 260 nm is an indication of denaturation of DNA
- In recombinant DNA Technology
- Renaturation following denaturation obeying base pairing rule is applicable in various hybridization and blotting techniques.

ORGANIZATION OF DNA

- Genome in the prokaryotes[Q] are loosely packed
- In eukaryotes DNA is well organized inside the nucleus.

Levels of Organization of DNA
- DNA double helix
- 10 nm chromatin fibril
- 30 nm chromatin fibril
- Nuclear Scaffold form (Interphase Chromosome)
  - Noncondensed loop
  - Condensed loop
- Metaphase Chromosome

DNA Double Helix

- First level of organization of DNA
- The characteristics are same as that of Watson-Crick model of DNA
- Diameter is 2 nm.

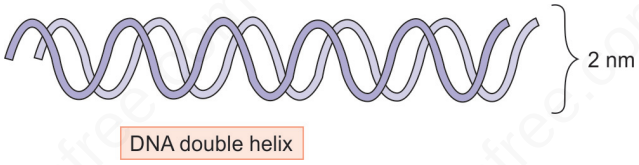


Fig. 9.2: DNA double helix

10 nm Chromatin Fibril

- Consist of nucleosomes separated by linker DNA
- Nucleosome is a nucleoprotein complex
- DNA double helix is wrapped nearly twice[Q] (exactly 1.75 times) over a histone octamer in left-handed helix to form a disk like structure
- Individual nucleosome are linked together by 30 bp segment called linker
- This gives a **Beads on a String appearance**[Q] on electron microscopy.

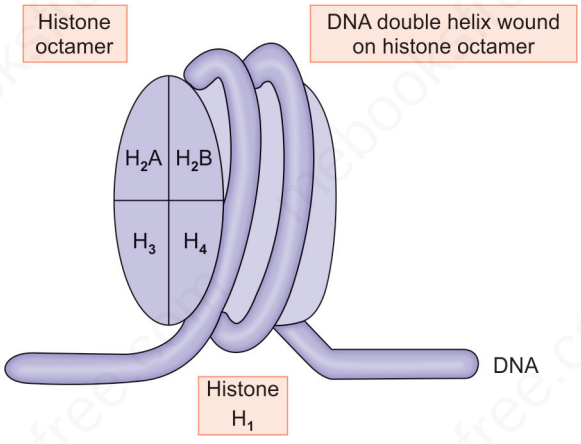


Fig. 9.3: Structure of nucleosome

Nucleosome at a Glance
- No of turns of DNA on Histone octamer—1.75 turns
- Direction of turn of the DNA over Histone—left handed.
- No of base pairs in 1.75 turn of DNA—145-150 bp
- Diameter of nucleosome—10 nm
- No of base pairs in the linker DNA—30 bp

HISTONES

- Most abundant chromatin Protein
- Small family of closely related basic proteins
- The carboxyl terminal two-third is hydrophobic, while amino terminal one-third is rich in basic amino acids like arginine and lysine
- Core histones are subject to at least six types of post-translational modifications
- They are highly conserved among the species.

Histones are divided into:
1. Core Histones
2. Linker Histones

Core Histones

- Core histones are H_2A, H_2B, H_3, and H_4
- They form histone octamer
- H_3 and H_4 forms tetramer, while H_2A and H_2B form dimers
- $(H_3\text{-}H_4)_2$ tetramer associate with two (H_2A-H_2B) dimers to form histone octamer
- H_2A and H_2B are Arginine[Q] rich
- H_3 and H_4 are Lysine rich.

Linker Histones

- H_1 histone which is seen in the linker region
- This is loosely bound[Q] to nucleosome.

Nonhistone Proteins

- They include enzymes involved in DNA replication and repair, RNA synthesis and processing
- Unlike histones, nonhistone proteins are acidic
- They are larger than histone proteins.

30 nm Chromatin Fibril (Solenoid)

Groups of nucleosome form 'DNA fibril'.
6 such DNA fibrils form 30 nm chromatin fibril.

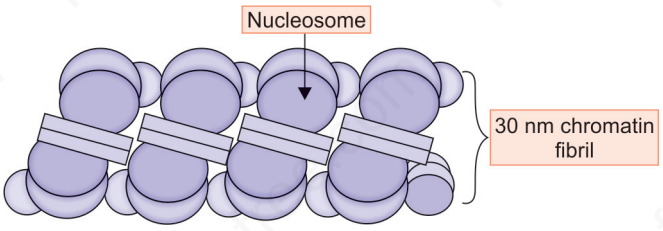


Fig. 9.4: Structure of chromatin fibril

Nuclear Scaffold Associated Form or Interphase Chromosome

- 30,000 to 100,000 bp loops or domains anchored in a scaffolding or supporting matrix, called nuclear matrix.
- Loops can be a condensed loop or non-condensed loops.

Euchromatin[Q] and Heterochromatin[Q]

Euchromatin

- Chromatin is less densely packed[Q]
- Transcriptionally active[Q]
- Chromatin stains less densely.[Q]

Heterochromatin

- Chromatin is densely packed[Q]
- Transcriptionally inactive[Q]
- Chromatin stains densely.[Q]

Two types of Heterochromatin

1. Constitutive Heterochromatin
   - Always condensed
   - Essentially inactive
   - Seen in Centromere and chromsomal ends of the telomere.
2. Facultative Heterochromatin
   - It is at times condensed, but at other times it is uncondensed and actively transcribed.

   For example: One of the X chromosome in mammalian female. The heterochromatic X chromosome decondenses during gametogenesis.

SUPERCOILING OF DNA

- DNA can be in relaxed or Superhelical
- Linear B-DNA is relaxed
- This is thermodynamically most favored, but biological activity of relaxed DNA, like replication, repair, etc. is reduced
- The biologically active form is superhelical, but it is topologically strained isomer.

Supercoils can be divided into:

1. Positive Supercoils
2. Negative Supercoils.

Positive Supercoils

Circular DNA twisted in the direction same as that original rotation creates positive supercoils or left-handed Superhelix. Such DNA is said to be overwound.

Negative Supercoils

Circular DNA twisted in the **direction opposite from the clockwise** turns of the **right-handed** double helix creates negative supercoils or right-handed helix. Such DNA is said to be **underwound**.

Functions of Supercoils

- Supercoiling promotes packing of DNA into compact structures
- Helps to generate regions with broken hydrogen bonds which facilitate DNA strand separation and facilitate replication, repair and recombination of the DNA.

Topoisomerase

- Nicking Resealing Enzyme
- Enzymes that can relax or insert supercoils
- Enzymes that relieve torsional strains in the DNA.

Topoisomerases can be of two types: Type I and Type II

Topoisomerase Type I

- Make transient single **stranded[Q] break** in a negatively supercoiled DNA double helix
- Phosphodiester bond is interrupted
- Topoisomerase remain bound to the phosphoryl group at incision site by a covalent bond, till the nick is resealed
- ATP is not needed.[Q]

There are two classes of Topoisomerases I

- Type IA and Type IB Topoisomerase

Type IA the tyrosine residue of the Topoisomerase is bound to 5′ phosphoryl group of nucleotide.

Type IB the tyrosine residue of the Topoisomerase is bound to 3′ phosphoryl group of nucleotide.

Topoisomerase Type II

- Dimeric enzyme that bind to double stranded DNA, make breaks in **both the strands[Q]** of DNA
- Can insert and remove supercoils
- ATP is needed.[Q]

Remember

- Bacterial DNA Gyrases[Q] is a subset of Topoisomerases type II
- All Topoisomerases type II relaxes supercoils in the DNA
- Bacterial DNA Gyrases are the only subset of type II topoisomerases that can add negative supercoils.

Some Important Prokaryotic Topoisomerases

Prokaryotic topoisomerases	Types	Functions
E coli Topoisomerase I	IA	Relaxes negatively supercoiled DNA
E coli Topoisomerase III	IA	Relaxes negatively supercoiled DNA
E coli Topoisomerase IV	II	Relaxes negatively supercoiled DNA
E coli DNA Gyrase	II	Introduces negative supercoils. Relaxes either positive or negative supercoils

Some Important Eukaryotic Topoisomerases

Eukaryotic topoisomerase	Types	Functions
Eukaryotic DNA Topoisomerase I	IB	Relaxes either negatively or positively coiled DNA
Eukaryotic DNA Topoisomerase II	II	Relaxes either negatively or positively coiled DNA
Eukaryotic DNA Topoisomerase III	IA	Possible role in recombination

Remember

The numbering given in the name of topoisomerase and the numbering in the types of Topoisomerases are different: For example, E coli Topoisomerase III belongs to Type IA Topoisomerase.

- Almost all E coli Topoisomerases relaxes negative supercoils.
- Only E coli DNA Gyrase introduces negative supercoils.
- All Eukaryotic DNA Topoisomerases relax negative and positive supercoils.

Bacterial Topoisomerases Inhibitors

1. Nalidixic Acid
2. Fluoroquinolones
3. Novobiocin.

Human Topoisomerase Inhibitors

Used in Cancer Chemotherapy.

Human Topoisomerases that inhibit Type I Topoisomerases

- Irinotecan
- Topotecan

Human Topoisomerases that inhibit Type II Topoisomerases

- Etoposide
- Adiramycin (Doxorubicin)
- Daunorubicin
- Idarubicin

CENTRAL DOGMA OF MOLECULAR BIOLOGY

Three process involved in Central Dogma of molecular biology are:

1. DNA replication (DNA to DNA)
2. Transcription (DNA to RNA)
3. Translation (RNA to protein)

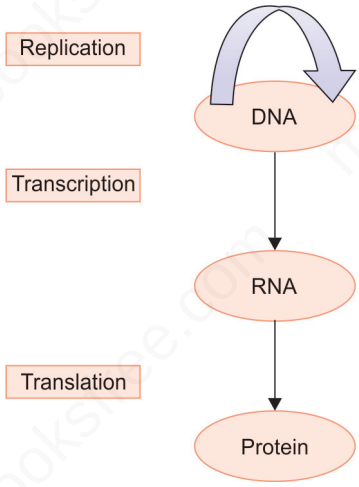


Fig. 9.5: Central dogma of molecular biology

Current Central Dogma of Molecular Biology

With the advances made by Human Genome Projects, the central dogma of molecular biology is changed.



- *Genome*: The complete set of genes of an organism.
- *Transcriptome*: The complete set of RNA transcripts produced by the genome of an organism.
- *Proteome*: The complete complement of proteins of an organism.

Numerals in Molecular Genetics

- Total number of chromosome in humans is 46 (23 pairs)
- The number of base pairs in haploid set of chromosome is 3.0×10^9 bp (3 billion bp)
- Percentage of exons in human genome is approximately 1.14% (1.5–2%)
- The number of protein coding genes in human genome is 20,687.
- The genes account for 10–15% of DNA.

REVIEW QUESTIONS

STRUCTURE OF DNA

1. Which of the following is false? (PGI 2002)

a. Ratio of A:T and G:C is approximately equal to 1:1
b. Ratio of A:G and T:C is approximately equal to 1:1
c. A + T = G + C
d. A + C = G + T
e. A + G = C + T

Ans. b, c, d. Ratio of A:G and T:C ..., A + T = G + C, A + C = G + T

Based on Chargaff's rule, Purines = Pyrimidines Adenine pair with Thymine and Guanine with Cytosine, hence, ratio of A:T & G:C is approximately equal to 1:1.

2. True about DNA structure: (PGI Nov 2010)

a. Purines are adenine and guanine and pyrimidines are uracil and cytosine
b. Watson and Crick discovered structure in 1973
c. Deoxyribose–phosphate backbone with bases stacked inside
d. Mainly consists of left-handed helix
e. 5′-3′ Phosphodiester bonding is present

Ans. c. Deoxyribose-phosphate backbone with bases stacked inside *(Ref: Harper 30/e page 359-362)*

Structure of DNA

Elucidated by Watson and Crick based on the X-ray diffraction picture taken by Rosalind Franklin in 1953 and got Nobel prize in 1962.

Salient Features of Watson–Crick Model of DNA

Right-handed Double stranded DNA helix.

Purines are Adenine and Guanine and Pyrimidines are Cytosine and Thymine.

Based on Pairing Rule:

- Adenine always pairs with Thymine
- Guanine pairs with Cytosine

Two strands are antiparallel.

The polarity of DNA is such that:

- One strand runs in 5′ to 3′ direction
- Other strand runs in 3′ to 5′ direction.

Hydrogen Bonding:

- Adenine pair with Thymine by 2 Hydrogen bonds. (A = T)
- Guanine pair with Cytosine by 3 Hydrogen bonds (G = C)

Grooves of the DNA

There are two types:

- Major groove
- Minor groove

Grooves act as sites of DNA-Protein interaction needed for regulation of gene expression.

3. If a sample of DNA if adenine is 23% what will be the amount of guanine present (PGI May 2013)

a. 23%
b. 25%
c. 46%
d. 27%
e. 54%

Ans. d. 27%

Based on Chargaff' rule:

- Purines = Pyrimidine
- No. of Adenine = No. of Thymine
- So Adenine + Thymine = 46%
- Guanine + Cytosine = 54%
- No. of Guanine = No. of Cytosine
- So the amount of Guanine = 54/2 = 27%

4. True statements about DNA structure: (PGI June 06)

a. All nucleotides are involved in linkage
b. Antiparallel
c. Parallel
d. Bases are perpendicular to DNA
e. Attached by hydrogen l bond

Ans. a, b, d and e. All nucleotides are …, Antiparallel, Bases are perpendicular … attatched by hydrogen bond *(Harper 30/e page 360)*

5. The two strands of DNA are held together by: (AIIMS Feb 97)

a. van der Waals bond
b. Hydrogen bond

c. Covalent bond
d. Ionic interaction

Ans. b. Hydrogen bond *(Harper 30/e page 360)*

6. Which form of DNA is predominantly seen: (AI 1996)

a. A
b. C
c. B
d. Z

Ans. c. B DNA *(Harper 30/e page 360)*

- Physiologically most common is B-DNA[Q]
- Under low salt and high degree of hydration B-DNA is usually found
- Under high salt concentration and low degree of hydration A-DNA is usually found
- The distance spanned by one turn of B-DNA is 3.4 nm (34 A^0)
- The width of the double helix in B-DNA is 2 nm (20A^0).

7. Chargaff rule state that: (Bihar 98, MP 04, UP 03)

a. A + G = T + C
b. A/T = G/C
c. A = U = T = G = C
d. A + T = G + C

Ans. a. A + G = T + C *(Harper 30/e page 360)*

Chargaff's rule is total number of Purines = Total number of Pyrimidines in a double stranded DNA.

8. A nucleic acid was analyzed and found to contain 32% adenine, 18% guanine, 17% cytosine and 33% thymine. The nucleic acid must be: (AIIMS May 06)

a. Single-stranded RNA
b. Single-stranded DNA
c. Double-stranded RNA
d. Double-stranded DNA

Ans: d. ds DNA *(Harper 30/e page 360)*

The above nucleic acid is obeying Chargaff's rule as the number of Purines (32 + 18) = No of Pyrimidines (17 + 33). Double stranded RNA is not the answer as it does not contain Thymine.

9. Triple bonds are found between which base pairs: (AI 2001)

a. A–T
b. C–G
c. A–G
d. C–T

Ans. b. C-G

- Three hydrogen bonds between C and G
- Two Hydrogen bonds between A and T.

10. Thermo-stability in DNA is contributed mostly by: (AI 1996)

a. A = T
b. G = C
c. Molecular base
d. Parallel arrangement

Ans. b. G = C

Factors Influencing Tm

1. *Base Composition.*
   More GC pairs more the Tm
2. *Salt concentration.*
   10-fold increase of monovalent cation concentration increases the Tm by 16.6°C.
3. Formamide[Q] destabilize hydrogen bond, hence decreases Tm.

11. At the physiological pH, the DNA molecules are: (AIIMS Nov 02)

a. Positively charged
b. Negatively charged
c. Neutral
d. Amphipathic

Ans. b. Negatively charged

DNA is negatively charged because of Phosphate group.

12. Total number of genes in a human being is: (Kerala 2001, CMC 04, WB 98)

a. 800,000
b. 50,000
c. 100,000
d. 30,000

Ans. d. 30,000

This is an old question, Now it is found that number of genes is approx 20,000.

13. Triplex DNA is due to: (AIIMS May 2011)

a. Hoogsteen pairing
b. Palindromic sequences
c. Large no. of guanosine repeats
d. Polypyrimidine tracts

Ans. a. Hoogsteen pairing

(Ref: Textbook of Biochemistry with clinical correlation Thomas M Devlin 7/e page 43)

NONCANONICAL DNA STRUCTURES

Triple-stranded DNA

Triple-stranded DNA is generated by the hydrogen bonding of a third strand into the major groove of B-DNA

Commonly seen in Polynucleotides, Poly (dA) and Poly (dT).

The third strand forms hydrogen bonds with another surface of the double helix through so-called Hoogsteen pairs.

14. About DNA which of the following is true (Jipmer 2014)

a. The nucleotide of one strand form bonds with nucleotide of opposite strand.
b. Cytosine and Uracil differ by one ribose sugar
c. The information from DNA is copied in the form of tRNA
d. Each nucleotide pair includes two purines.

Ans: a. The nucleotide of one strand form bonds with nucleotide of opposite strand

- One strand af DNA join with other strand of DNA by means of Hydrogen bond between the bases.
- Cytosine and uracil differ by amino group.
- The information in the DNA is copied in the form of mRNA
- Each nucleotide pair includes one purine and one pyrimidine.

15. Which model of DNA was discovered by Watson and Crick? (NBE Pattern Q)

a. A-DNA
b. B-DNA
c. C-DNA
d. Z-DNA

Ans. b. B-DNA

Organization of DNA

16. Total number of base pairs in human haploid set of chromosome (Ker 2007)

a. 3 million
b. 3 billion
c. 33 billion
d. 5 million

Ans. b. 3 billion (3×10^9) *(Harper 30/e page 377) (Harrison 19/e page 427)*

- Human haploid genome of each cell consist of 3×10^9 bp (3 billion bp)
- Current estimates predict 20,687 protein coding genes
- Exome constitutes 1.14% of genome
- SNPs estimated is 10 million.

17. Proteins seen in chromosomes are called: (Ker 2006)

a. Nucleotides
b. Histones
c. Apoproteins
d. Glycoproteins

Ans. b. Histones *(Ref: Harper 30/e page 371)*

Histones are the most abundant histone proteins.

18. Euchromatin is the region of DNA that is relatively: (AI 2006)

a. Uncondensed
b. Condensed
c. Overcondensed
d. Partially condensed

Ans. a. Uncondensed *(Ref: Harper 30/e page 374)*

Euchromatin and Heterochromatin

Euchromatin

- Chromatin is less densely packed
- Transcriptionally active
- Chromatin stains less densely

Heterochromatin

- Chromatin is densely packed
- Transcriptionally inactive
- Chromatin stains densely

Two Types of Heterochromatin

1. Constitutive Heterochromatin:
   - Always condensed
   - Essentially inactive
   - Seen in centromere and chromsomal ends of the telomere.
2. Facultative Heterochromatin:
   - Is at times condensed, but at other times it is uncondensed and actively transcribed, e.g. one of the X chromosome in mammalian female.
   - The heterochromatic X chromosome decondenses during gametogenesis.

19. The long and short arms of chromosomes are designated respectively as (AI 2006)

a. P and q arms
b. M and q arms

c. q and p arms
d. I and s arms

Ans. c. q and p arms

(Emery's Elements of Medical Genetics 13/e page 31)

20. True about Histone Proteins (PGI May 2012)

a. Ribonucleoprotein
b. Present inside the nucleus
c. Acidic
d. Basic
e. Glycoprotein

Ans. b, d. Present inside .., basic

(Ref: Harper 29/e page 371)

Histones

- Most abundant Chromatin Protein
- Small family of closely related basic Proteins

There are 5 classes of Histones:

- H_1, H_2A, H_2B, H_3, and H_4

Core Histones are:

- $H_3 + H_4 + H_2A + H_2B$
- They form Histone Octamer.

Linker Histones

- H_1 histone which is seen in the linker region.
- Loosely bound to nucleosome.

Nonhistone proteins

- Most of which are acidic and larger than histones
- The nonhistone proteins include enzymes involved in DNA replication and repair, and the proteins involved in RNA synthesis, processing, and transport to the cytoplasm.

21. Y-chromosome is: (AIIMS May 2008)

a. Metacentric
b. Submetacentric
c. Acrocentric
d. Longer than the X-chromosome

Ans. c. Acrocentric

(Ref: Emery's Elements of Medical Genetics page 30, 31)

Karyotype

Arrangement of chromosome in the decreasing order of length.

DIFFERENT GROUPS OF CHROMOSOME

GROUPS	CHROMOSOME	DESCRIPTION
A	1–3	Largest; 1 & 3 Metacentric 2 -Submetacentric
B	4,5	Large Submetacentric
C	6–12, X	Medium size, Submetacentric
D	13–15	Medium size, Acrocentric with satellite
E	16–18	Small; 16 is metacentric but 17 & 18 are Submetacentric
F	19–20	Small Metacentric
G	21, 22, Y	Small Acrocentric

Points to Ponder

- X Chromosome is Submetacentric (C)
- Y Chromosome is Small Acrocentric (G)
- Most common is Submetacentric
- Humans lack Telocentric Chromosome

22. Nucleosome consists of: (PGI May 2010)

a. Histone
b. DNA
c. RNA
d. DNA and RNA both
e. Carbohydrate

Ans. a, b. Histone, DNA *(Ref: Harper 30/e page 371)*

Nucleosome

- Nucleoprotein complex
- DNA double helix is wrapped nearly twice over a histone octamer in left handed helix to form a disk like structure.
- Individual nucleosome are linked together by 30 bp segment called linker. This gives a **Beads on a String appearance** on electron microscopy.

23. Component of chromosome are: (PGI Dec 03)

a. DNA
b. tRNA
c. mRNA
d. rRNA
e. Histones

Ans. a, e. DNA, Histones *(Harper 30/e page 371)*

24. The protein rich in basic amino acids, which functions in the packaging of DNA in chromosome, is: (AI 2003)

a. Histones
b. Collagen

c. Hyaluronic acid binding protein
d. Fibrinogen

Ans. a. Histones *(Harper 30/e page 371)*

25. Random inactivation of X chromosome is:
a. Lyonization
b. Allelic Exclusion
c. Randomization
d. Genomic imprinting

Ans. a. Lyonization

Two factors that are peculiar to the sex chromosomes: (1) lyonization or inactivation of all but one X chromosome and (2) the modest amount of genetic material carried by the Y chromosome.

In 1961, Lyon outlined the idea of X-inactivation, now commonly known as the Lyon hypothesis. It states that (1) only one of the X chromosomes is genetically active, (2) the other X of either maternal or paternal origin undergoes heteropyknosis and is rendered inactive, (3) inactivation of either the maternal or paternal X occurs at random among all the cells of the blastocyst on or about day 16 of embryonic life, and (4) inactivation of the same X chromosome persists in all the cells derived from each precursor cell.

The inactive X can be seen in the interphase nucleus as a darkly staining small mass in contact with the nuclear membrane known as the Barr body, or X chromatin. The molecular basis of X inactivation involves a unique gene called XIST, whose product is a noncoding RNA that is retained in the nucleus, where it 'coats' the X chromosome that it is transcribed from and initiates a gene-silencing process by chromatin modification and DNA methylation. The XIST allele is switched off in the active X.

26. In the entire genome, the coding DNA constitutes how much: (AIIMS 2014 May)
a. 0.1
b. 0.02
c. 0.25
d. 0.4

Ans. b. 0.02 In the entire genome, the coding DNA constitutes 1.5–2% (approx 1.14% according to Harrison 19/e and Harper 30/e).

27. True about DNA hyperchromatism (PGI Nov 2013)
a. It is increase of absorbance
b. Measured by absorbance at 260 nm (in a spectrophotometer)
c. It occurs when the DNA duplex is denatured
d. Double stranded DNA is more hyperchromic than ssDNA

Ans. a, b and c. It is increase of absorbance, Measured by absorbance at 260 nm (in a spectrophotometer), it occurs when the DNA duplex is denatured.

During denaturation of DNA, there is increased in absorbance at 260 nm, measured by Spectrophotometry. This is called hyperchromicity.

ss DNA is more hyperchromic than ds DNA.

10 DNA Replication

Topics Included

- Definition
- Enzymes Involved in the DNA Replication
- DNA Polymerases
- Steps of DNA Replication
- DNA Repair Mechanisms

DNA REPLICATION

Definition

The process by which copying of base sequence present in the parent strand to daughter strand, thereby passing the genetic information from parent to progeny is called Replication.

Salient Features of DNA Replication

- Occurs in the S Phase of the cell cycle
- DNA strands separate and each acts as template strand on which complementary strand is synthesized
- Base pairing rule is obeyed
- Semiconservative nature-Proved by Meselson and Stahl Experiment
  - Half of the parent strand is conserved in the daughter DNA
- New Strand is synthesized always in 5′ to 3′ direction
- Overall DNA replication is bidirectional
- Synthesis of DNA in both strands are not similar
  - Leading Strand: The strand which DNA is continuously polymerized
  - Lagging Strand: The strand which is DNA is discontinuously polymerized.

Enzymes Involved in the DNA Replication

- **Topoisomerases**
  - Relieve torsional strain that results from helicase-induced unwinding of DNA
  - Nicking Resealing Enzyme
- **Helicase:** ATP driven processive unwinding of DNA
- **Single Strand Binding (SSB)** protein prevents premature reannealing of ds DNA.
- **DNA Primase:**
  - Initiates synthesis of RNA primers

  Special class of DNA dependent RNA Polymerase
- **DNA Polymerase:** Catalyse the chemical reaction of DNA Polymerization. Synthesize DNA only in 5′ to 3′ direction
- **DNA Ligase:** Seals the single strand nick between the nascent chain and Okazaki fragments on lagging strand.

DNA POLYMERASES[Q]

- Enzymes that catalyse Deoxyribonucleotide polymerization
- The initiation of DNA synthesis by DNA Polymerase always require priming by a short length of RNA, called Primer.

Three important properties of DNA Polymerase Complex

1. **Chain elongation[Q]:** Chain elongation accounts for the rate (in nucleotides per second; nucleotides/s) at which polymerization occurs.

   Rate of chain elongation of DNA Pol III is 20-50 nucleotides/second.
2. **Processivity[Q]:** Processivity is an expression of the number of nucleotides added to the nascent

chain before the polymerase disengages from the template. Processivity of DNA Pol III is 100 to >50,000 nucleotides.

3. **Proofreading[Q]:** The proofreading function identifies copying errors and corrects them.

- Proofreading function needs 3' to 5' exonuclease activity
- Repair function needs 5' to 3' exonuclease activity.

Prokaryotic DNA Polymerase

Three types of Prokaryotic DNA Polymerase

- Pol I
- Pol II
- Pol III

Prokaryotic DNA polymerase	Function
Pol I	Removal of Primers and Gap filling on lagging strand DNA Proofreading DNA Repair Recombination
Pol II	DNA proofreading and repair
Pol III	Processive, leading strand synthesis Synthesis of Okazaki fragments

Role of DNA Sliding Clamp

DNA Polymerase III associate with two identical β subunits of DNA Sliding 'clamp' which increases the Pol III–DNA stability, processivity and rate of chain elongation.

Bacterial DNA Polymerase—at a Glance

- Main replication DNA Polymerase is DNA Polymerase III
- DNA Polymerase with highest rate of chain elongation (Most Processive)[Q] is Pol III
- DNA Polymerase with proofreading activity[Q]: Pol I, Pol II and Pol III
- DNA Polymerase with repair activity: Pol I and Pol II
- DNA Polymerase which fills the gap in the lagging strand is Pol I
- DNA Polymerase which polymerises Okazaki fragments Pol III
- DNA Polymerase which synthesizes leading strand Pol III
- Kornberg's enzyme is DNAP I, as it is discovered by Arthur Kornberg
- Arthur Kornberg described the existence of DNA Polymerase I in *E coli*
- Klenow Fragment[Q]: DNA Polymerase in which 5' to 3' exonuclease activity is removed.

Eukaryotic DNA Polymerase

Mainly five types of Eukaryotic DNA Polymerase

- DNAP α
- DNAP β
- DNAP γ
- DNAP δ
- DNAP ε

Eukaryotic DNA polymerase	Function
DNAP alpha	Primase
DNAP beta	DNA repair
DNAP gamma	Mitochondrial DNA synthesis
DNAP delta	Lagging strand synthesis
DNAP epsilon	Leading strand synthesis

Remember

- Eukaryotic DNA Polymerase involved in DNA repair are DNA Polymerase delta (δ) and eta (η).

Steps of DNA Replication

- **Identification of the origins of replication**

  Fixed points on the chromosome where replication begins are called Ori
  - In *E coli*-ori C
  - In Bacteriophage λ-ori λ
  - In Yeast-Autonomous Replicating Sequence (ARS)
  - In humans similar to Yeast

Single ori in bacteria

Multiple ori present in eukaryotes

There is an AT rich sequence adjacent to ori facilitating DNA unwinding.

In eukaryotes ~80 bp AT rich sequence called DNA UNWINDING ELEMENT (DUE).

- **Ori+ ds DNA binding Protein (DNA A) opens the DNA duplex**
  - Unwinding (denaturation) of ds DNA to provide an ssDNA template
  - Ori + ds binding protein causes local denaturation of DNA.
  - This facilitates the further unwinding of DNA by **Helicase**.

Role of Single Strand Binding Protein (SSB)

- Prevents the re annealing of the separated DNA strands.
- Human SSBs are called Replication Protein A (RPA)
- **Formation of the replication fork**
  - Unwinding of DNA forms replication bubble
  - A pair of replication fork is replication bubble

  Synthesis of RNA primer by Primase synthesize 100–200 length Ribonucleotides.

- DNA G is the primase in case of Prokaryotes
- DNAP α[Q] has primase activity in case of eukaryotes

- **Initiation of DNA synthesis and elongation**

Two strands are synthesized in different manner[Q]

On 3′ ----> 5′ Strand (Leading Strand) (Continuous Strand) (Forward Strand)

Synthesized in continuous[Q] manner on the 3′ hydroxyl end of RNA primer in 5′ to 3′ direction

- In Prokaryotes by DNA Polymerase III
- In Eukaryotes by DNA Polymerase ε

On 5′----> 3′ Strand (Lagging Strand) (Discontinuous Strand) (Retrograde Strand)

Synthesized in discontinuous[Q] manner.

- Small fragments of DNA are added in short spurts called **Okazaki Fragment**[Q] synthesized in 5′ ---- 3′ direction.
  - By DNAP III in prokaryotes
  - By DNAP δ in eukaryotes
  - Length of Okazaki fragments in Prokaryotes is 1000 to 2000 nucleotides
  - Length of Okazaki fragments in Eukaryotes is 100 to 250 nucleotides.
- Removal of RNA Primers and gap filling of the lagging strand.
  - In Prokaryotes—Removal of RNA Primer and Gap filling by **DNA polymerase-I**
  - In Eukaryotes-**RNAse H** removes the primer
  - DNA P δ fills the gap, where RNA Primer is removed.
- Sealing the nick following gap filling.
  - **DNA ligase:** Seals the single strand nick between the nascent chain and Okazaki fragments on lagging strand.[Q]

- Length of Okazaki fragments in Prokaryotes is 1000 to 2000 nucleotides
- Length of Okazaki fragments in eukaryotes is 100 to 250 nucleotides
- Time taken for replication in bacteria is 30 minutes.
- Time taken for replication of entire human genome is 9 hours.

Replisome

Multimeric proteins that assemble in the replication fork are called Replisome.

It includes:

- DNA helicase
- Primase
- DNA polymerase
- Single strand binding proteins

Contd...

Contd...

Primosome

Mobile complex between helicase and primase.

Telomere[Q] and Telomerase[Q]

- On the 5′ end of the newly synthesized linear DNA, RNA primer is removed by RNAase H
- This leaves a gap at the 5′ end of daughter strand.
- In other words 3′ end of the leading strand is not synthesized.
- This results in shortening of DNA with each cell division.
- This is prevented by presence of Telomere[Q] and Telomerase.[Q]

Telomeres

- The ends of chromosome contain structures called telomeres
- Telomeres consist of short T-G repeats
- Human telomeres have variable number of tandem repeats of the sequence 5′ TTAGGG-3′.

Telomerase (Telomere Terminal Transferase)

- Enzyme which prevents shortening of DNA
- Has an intrinsic RNA primer[Q]
- Has Reverse Transcriptase[Q] (RNA Dependent DNA Polymerase) activity.
- Present in Germ line[Q], stem cells[Q], most cancer cells[Q].
- Absent from most somatic cells[Q].

Clinical Significance of Telomerase

- Absence of Telomerase lead to premature ageing
- In cancer cells increased Telomerase activity
- Telomere shortening is associated with ageing, malignancy
- Telomerase has become an attractive target for cancer chemotherapy and drug development.

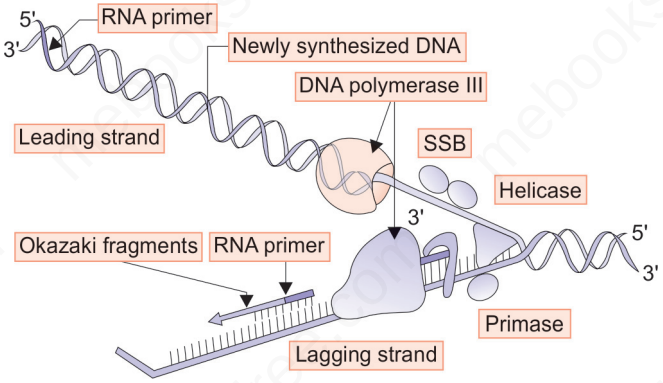


Fig. 10.1: DNA replication

Reverse Transcriptase
- Temin and Baltimore isolated this enzyme in 1970
- They are RNA Dependent DNA Polymerase
- Synthesize new DNA strand with RNA as template
- Thus, they reverse Central dogma of molecular genetics
- These enzymes are important in RNA Viruses like Retroviruses
- Telomerase has reverse transcriptase activity.

DNA REPAIR MECHANISMS

- DNA is subjected to a huge array of chemical, physical, and biological assaults on a daily basis
- Repair of damaged DNA is critical for maintaining genomic integrity and thereby preventing the propagation of mutations
- Eukaryotic cells contain five major DNA repair pathways.

The mechanisms of DNA repair include:
- Nucleotide Excision Repair (NER)
- Mismatch Repair (MMR)
- Base Excision Repair (BER)
- Homologous Recombination (HR)
- Nonhomologous End-Joining (NHEJ) repair.

DNA damaging agents	Defects in DNA	Repair mechanism	Disorder associated
1. Ionizing Radiations 2. X-rays[Q] 3. Antitumor drugs	1. Double Strand Breaks[Q] 2. Single Strand Breaks 3. Intrastrand cross links 4. Interstrand cross links	Nonhomologous End Joining (NHEJ) Homologous Recombination (HR)	Severe Combined Immunodeficiency (SCID) Ataxia Telangectasia Like Disorder (ATLD) Nijimen break Syndrome (NBS) Bloom's Syndrome (BS)

Contd...

DNA damaging agents	Defects in DNA	Repair mechanism	Disorder associated
			Werner Syndrome (WS) Rothmund-Thomson Syndrome (RTS) Breast Cancer Susceptibility (BRCA 1, BRCA 2)
1. UV light[Q] 2. Chemicals	1. Bulky Adducts 2. Pyrimidine Dimers[Q]	Nucleotide Excision Repair (NER)[Q]	Xeroderma Pigmentosa[Q] (XP) Cockayne Syndrome (SS) Trichothiodystrophy (TTD)
1. Oxygen radicals 2. Alkylating agents	1. Abasic Sites 2. Single strand breaks 3. 8 oxoguanine lesions	Base Excision Repair (BER)	MUTYH–associated Polyposis (MAP)
1. Replication errors	1. Bases mismatch 2. Insertion 3. Deletion	Mismatch repair (MMR)	Hereditary nonpolyposis Colorectal Cancer (HNPCC)[Q] (Lynch syndrome)

Double Strand Break Repair Mechanisms (DSB)

- They are Homologous recombination (HR) and non Homologous End Joining Repair (NHEJ)

Homologous recombination	Nonhomologous end joining repair
Major mechanism of DSB repair in yeast	Major mechanism of DSB repair mammals
Takes place between homologous chromosomes	Does not need a homologous Chromosome
Takes place before cell enter mitosis (S and G2/M phase)	Takes place before cell enter mitosis (G0/G1 phase)

REVIEW QUESTIONS

DNA REPLICATION

1. False statements is/are: (PGI May 2011)

a. In leading strands DNA is synthesized continuously
b. Multiple origins of replication are possible for bacteria
c. DNA replication proceeds in one direction
d. Lagging strand stick by RNA primase
e. DNA polymerase III-processive leading strand synthesis

Ans. b, c, d. *(Harper 30/e page 381-387)*

- Multiple ori in eukaryotes

- DNA replication is bidirectional
- Lagging strand stick by DNA Ligase

DNA Replication

Salient Features of DNA Replication

- Occurs in the S Phase of the cell cycle
- Each DNA Stand separate and each acts as template strand on which complementary strand is synthesized
- Base pairing rule is obeyed
- Semi conservative nature-Proved by Meselson and Stahl Experiment
- New Strand is synthesized in the 5′ to 3′ direction
- Synthesis of DNA in both strands are not similar
  - **Leading strand:** The strand which DNA is continuously polymerized
  - **Lagging Strand:** The strand which is DNA is discontinuously polymerized (Semi discontinuous)
- Replication proceeds from multiple origins in each chromosome in eukaryotes including humans (a total of as many as 100 in humans)
- Replication obeys polarity
- **Replication occurs in both directions** along all of the chromosomes, i.e. bidirectional in prokaryotes and eukaryotes
- Both strands are replicated simultaneously
- Replication process generates **'replication bubbles'.**

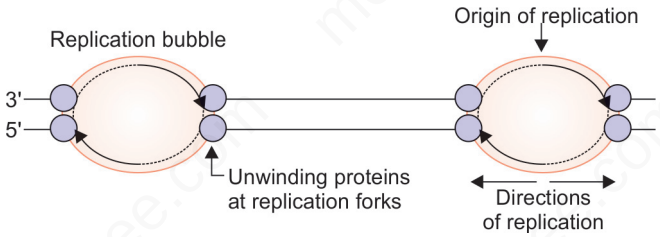


Fig. 10.2: Direction of replication

2. True about Eukaryotic DNA replication compared to prokaryotic: (PGI May 2013)

a. Conservative
b. Semi conservative
c. Unidirectional
d. Bidirectional
e. Semi discontinuous

Ans. b, d, e. Semi conservative, Bidirectional, Semi discontinuous. *(Harper 30/e page 381-387)*

This questions means the common features between eukaryotic and prokaryotic DNA replication.

3. Incorrect statements are: (PGI Nov 2010)

a. T4 DNA polymerase has 3′->5′ exonuclease activity
b. Klenow fragment of DNA polymerase I function is almost similar to T4 DNA polymerase
c. Restriction endonuclease cut DNA chains at specific location
d. Endonuclease cut DNA at 5′ terminus
e. Right-handed helix of DNA is more common

Ans. d. Endonuclease cut DNA at 5′ terminus.

Endonuclease cut the DNA from within

Exonuclease cuts the DNA from ends

T4 DNA Polymerase similar to Klenow polymerase.

4. Which DNA polymerase is involved in repair of mammalian DNA: (PGI June 2009)

a. Alpha
b. Beta
c. Gamma
d. Epsilon
e. Delta

Ans. b. Beta *(Ref: Harper 30/e page 383)*

Eukaryotic DNA Polymerase

Mainly five types of Eukaryotic DNA Polymerase

- DNAP α
- DNAP β
- DNAP γ
- DNAP δ
- DNAP ε

Eukaryotic DNA polymerase	Function
DNAP alpha	Primase
DNAP beta	DNA repair[Q]
DNAP gamma	Mitochondrial DNA synthesis
DNAP delta	Lagging strand synthesis
DNAP epsilon	Leading strand synthesis

Prokaryotic DNA Polymerase

Three types of Prokaryotic DNA Polymerase

- Pol I
- Pol II
- Pol III

Prokaryotic DNA Polymerase	Function
Pol I	Gap filling following DNA replication, repair, and recombination

Contd...

Contd...

Prokaryotic DNA Polymerase	Function
Pol II	DNA proofreading and repair
Pol III	Processive, leading strand synthesis, Okazaki fragment synthesis

5. The gaps between segment of DNA on the lagging stand produced by restriction enzymes are joined/sealed by: (AI 2009)

a. DNA Ligases
b. DNA Helicase
c. DNA Topoisomerase
d. DNA Phosphorylase

Ans. a. DNA Ligase *(Ref: Harper 30/e page 381)*

6. During replication of DNA, which one of the following enzymes polymerizes the Okazaki fragments? (AI 2006)

a. DNA Polymerase I
b. DNA Polymerase II
c. DNA Polymerase III
d. RNA Polymerase I

Ans. c. DNA Polymerase III *(Harper 30/e page 381-387)*

DNA Synthesis

On 5′----> 3′ Strand (Lagging Strand) (Discontinuous Strand) (Retrograde Strand)

Synthesized in discontinuous manner

Small fragments of DNA are in short spurts of 100–250 nucleotide (1000–2000 bp in prokaryotes) called Okazaki Fragment synthesized in 5′ --- 3′ direction.

- By DNAP III in prokaryotes.
- By DNAP δ in eukaryotes.

7. All of the following cell types contain the enzyme telomerase which protects the length of telomerase at the end of chromosomes, except: (AI 2006)

a. Germinal
b. Somatic
c. Hemopoietic
d. Tumor

Ans. b. Somatic *(Harper 30/e page 374)*

Telomere and Telomerase

- On the 3′ end of the linear DNA, RNA primer is removed by RNAaseH, which leaves a gap at the 3′ end
- Thus 5′ end of the new strand is not synthesized
- This results in shortening of DNA with each cell division
- This is prevented by presence of Telomere and Telomerase.

Telomeres

- Are tandem repeats of simple sequence on 3′ end of the parent DNA
- In humans repeats are (TTAGG)n.

Telomerase (Telomere Terminal Transferase)

- Enzyme which prevents shortening of DNA
- Has an intrinsic RNA primer
- Has Reverse Transcriptase (RNA Dependent DNA Polymerase) activity
- Present in Germ line, stem cells, most cancer cells
- Absent from most somatic cells.

Clinical Significance of Telomerase

- Absence of Telomerase lead to premature aging
- In Cancer cells increased Telomerase activity
- Telomerase has become an attractive target for cancer chemotherapy and drug development.

8. DNA Polymerase with both replication and repair function is: (Ker 2009)

a. I
b. II
c. III
d. None of the above

Ans. a. I and **b.** II *(Harper 30/e page 383)*

9. Radiolabelled DNA was allowed to replicate twice in a nonradioactive environment. Which of the following is true? (Ker 2008)

a. All the strands will have radioactivity
b. Half of the DNA will have no radioactivity
c. No strands will have radioactivity
d. Three–fourth of the DNA replicated will have radioactivity.

Ans. b. Half of the DNA will have no radioactivity *(Harper 30/e page 381-387)*

- Semi conservative nature of DNA Replication proved by Meselson and Stahl states that half of the parent strand is conserved during replication in the daughter strand
- After one replication all the DNA will have radioactivity
- After two replication half of the DNA will have radioactivity.

10. In which of the following phase, DNA doubling occurs: (Ker 2006)

a. Gl phase
b. S phase
c. G2 phase
d. M phase

Ans. b. S Phase *(Harper 30/e page 381-387)*

11. Unwinding Enzyme in DNA synthesis:

a. Helicase
b. Primase
c. DNA Polymerase
d. Transcriptase

Ans. a. Helicase *(Ref: Harper 30/e page 382)*

Classes of Proteins involved in DNA Replication	
Protein	**Function**
DNA polymerases	Deoxynucleotide polymerization
Helicases	Processive unwinding of DNA
Topoisomerases	Relieve torsional strain that results from helicase-induced unwinding
DNA primase	Initiates synthesis of RNA primers
Single-strand binding proteins	Prevent premature reannealing of dsDNA
DNA ligase	Seals the single strand nick between the nascent chain and Okazaki fragments on lagging strand

12. True about telomerase or telomere is/are: (PGI Dec 03)

a. They are present at the ends of eukaryotic chromosome
b. Increased telomerase activity favors cancer cells
c. DNA dependent RNA polymerase
d. DNA polymerase

Ans. a, b. They are present at the ends of …, increased telomerase… *(Harper 30/e page 374)*

- Telomeres are present in the ends of eukaryotic chromosomes.
- Telomeres consist of TG repeats.
- Telomere shortening has been associated with malignant transformation and aging
- Telomerase, multisubunit RNA template containing RNA Dependent DNA Polymerase (Reverse Transcriptases).
- Enzyme responsible for Telomere synthesis and maintaining the length of telomere.

13. Action of telomerase is: (CUPGEE 2011)

a. DNA repair
b. Longevity of cell
c. Breakdown of telomere
d. None

Ans. b. Longevity of cell-aging *(Harper 30/e page 374)*

14. Ends of chromosomes replicated by: (PGI Dec 06)

a. Telomerase
b. Centromere
c. Restriction endonuclease
d. Exonuclease

Ans. a. Telomerase *(Harper 30/e page 374)*

15. Highly repetitive DNA is seen in: (PGI June 3)

a. Cloning of DNA
b. Microsatellite DNA
c. Telomere
d. Centromere

Ans. c, d. Telomere and Centromere *(Harper 30/e page 377)*

- In human DNA, at least 30% of genome consist of repetitive sequence
- These sequences are clustered in the centromere and and telomere
- They are transcriptionally inactive
- They are mostly having structural role in the chromosome.

16. Which enzymatic mutation is responsible for immortality of cancer cells: (AIIMS Nov 01)

a. DNA reverse transcriptase
b. RNA polymerase
c. Telomerase
d. DNA polymerase

Ans. c. Telomerase *(Robbins 9/e page 288)*

Telomerase is one of several factors that contribute to the endless replicative capacity (the immortalization) of cancer cells.

17. Okazaki fragments are formed during the synthesis of: (AI 08)

a. dsDNA
b. ssDNA
c. mRNA
d. tRNA

Ans. a. ds DNA

18. Correct sequence of enzymes required for DNA formation is: (PGI June 01)

a. DNA polymerase → protein unwinding enzyme → DNA ligase → DNA Isomerase → Polymerase I
b. Protein unwinding enzyme → polymerase I → DNA ligase → DNA isomerase → DNA polymerase
c. RNA polymerase → DNA polymerase III → DNA polymerase I → DNA ligase
d. RNA polymerase → DNA polymerase III → DNA ligase → exonuclease → DNA polymerase I

Ans. c. RNA Polymerase → DNA polymerase III → DNA polymerase I → DNA ligase *(Harper 30/e page 381)*

- The correct sequence of enzymes is Helicase, Primase, DNA Polymerase III, DNA Polymerase I and on lagging strand, DNA ligase
- Helicase, Primase, DNA Polymerase III on leading strand.

19. True about DNA polymerase in eukaryotes: (PGI June 08)

a. Components are α, β, γ, δ, ε
b. β associated with repair
c. γ associated with repair
d. δ associated with synthesis of mitochondria DNA
e. α is abundant amount

Ans. a, b. Components are ..., β associated with ... *(Harper 30/e page 381)*

Eukaryotic DNA polymerase	Function
DNAP alpha	Primase
DNAP beta	DNA repair
DNAP gamma	Mitochondrial DNA synthesis
DNAP delta	Lagging Strand Synthesis
DNAP epsilon	Leading Strand Synthesis

20. DNA polymerase have: (PGI June 03)

a. 3′–5′ polymerase activity
b. 5′–3′ polymerase activity
c. 3′–5′ exonuclease activity
d. 5′–3′ exonuclease activity

Ans. b, c, d. 5′–3′ polymerase activity, 3′–5′ exonuclease activity, 5′–3′ exonuclease activity *Harper 30/e page 382*

DNA Polymerase have 5′ to 3′ Polymerase activity, 3′–5′ exonuclease (Proofreading) and 5′–3′exonuclease activity (repair) activity.

DNA Repair

21. Xeroderma pigmentosa is due to: (Ker 2006)

a. Base excision defect
b. Nucleotide excision defect
c. SOS repair defect
d. Cross linking defect

Ans. b. Nucleotide excision defect *(Harper 30/e page 389)*

DNA damaging agents	Defects in DNA	Repair Mechanism	Disorder associated
• Ionizing Radiations • X-rays • Antitumor drugs	• Double Strand Breaks • Single Strand Breaks • Intrastrand cross links • Interstrand cross links	Nonhomologous End Joining (NHEJ) Homologous Recombination (HR)	Severe Combined Immunodeficiency (SCID) Ataxia Telangiectasia Like Disorder Nijmen Break Syndrome Bloom's Syndrome, Werner Syndrome, Rothmund-Thomson Syndrome Breast Cancer Susceptibility (BRCA 1, BRCA 2)
• UV light • Chemicals	• Bulky Adducts • Pyrimidine Dimers	Nucleotide Excision Repair (NER)	Xeroderma Pigmentosa Cockayne Syndrome Trichothiodystrophy
• Oxygen radicals • Alkylating agents	• Abasic Sites • Single strand breaks • 8 oxoguanine lesions	Base Excision Repair (BER)	MUTYH – associated Polyposis
• Replication errors	• Bases mismatch • Insertion • Deletion	Mismatch repair (MMR)	Hereditary nonpolyposis Colorectal Cancer (HNPCC)

22. UV light damage to the DNA leads to: (PGI Dec 05)

a. Formation of pyrimidine dimers
b. No damage to DNA
c. DNA hydrolysis
d. Double stranded breaks

Ans. a. Formation of pyrimidine dimers

(Harper 30/e page 390)

DNA lesions formed by UV light damage are Bulky adducts and Pyrimidine Dimers.

23. Excessive ultraviolet (UV) radiation is harmful to life. The damage caused to the biological system by ultraviolet radiation I by: (AIIMS May 04)

a. Inhibition of DNA synthesis
b. Formation of thymidine dimers
c. Ionization
d. DNA fragmentation

Ans. b. Formation of thymidine dimers

(Harper 30/e page 390)

24. The primary defect in Xeroderma pigmentosa is: (AI 2000)

a. Formation of thymidine dimers
b. Poly ADP ribose polymerase is defective
c. Exonuclease is defective
d. Formation of adenine dimers

Ans. a. Formation of Thymidine dimers

(Harper 30/e page 390)

- UV light radiation causes Bulky adducts and Pyrimidine dimers (Most common is Thymidine dimers)
- This is repaired by Nucleotide excision repair (NER)
- Defect in NER leads to Xeroderma Pigmentosa.

25. Which of the following is true regarding DNA double-strand breaks repair pathway:

a. Homologous recombination require a long homologous sequence to guide repair
b. Nonhomologous end-joining does not require a long homologous sequence to guide repair
c. Homologous recombination repairs DNA before the cell enters mitosis
d. Nonhomologous end-joining repairs DNA before the cell enters mitosis
e. Nonhomologous end-joining is prominent DSB repair mechanism in mammals.

Ans. a, b, c, d, e.

Double Strand Break Repair Mechanisms (DSB). They are Homologous recombination (HR) and Nonhomologous End Joining Repair (NHEJ).

Homologous recombination	Nonhomologous end joining repair
Major mechanism of DSB repair in yeast	Major mechanism of DSB repair mammals
Takes place between homologous chromosomes	Does not need a homologous Chromosome
Takes place before cell enter mitosis (S & G2/M phase)	Takes place before cell enter mitosis (G0/G1 phase)

11 Transcription

Topics Included

- Definition and Salient Features
- Steps of Transcription
- Post-transcriptional Modification
- Different Classes of RNA

TRANSCRIPTION

Definition

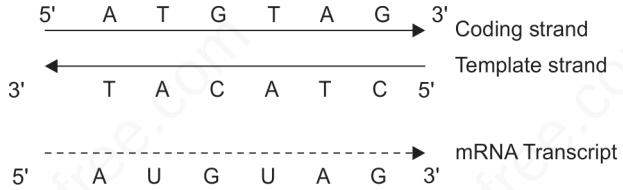
The process by which RNA is synthesized from the DNA is called Transcription.

Salient Features of Transcription

Template Strand and Coding Strand

The strand that is transcribed or copied to the mRNA is referred to as Template strand or Nonsense strand.

The opposite strand is referred to as Coding strand or Non-template strand or Sense Strand.



The nontemplate strand is called coding strand

- Primary transcript is complementary to the template strand.
- Hence, the coding strand contains the same base sequence in the nascent mRNA except in the case of Thymine replaced by Uracil
- Hence, nontemplate strand is called coding strand.

RNA Polymerase (RNAP)

They are DNA dependent RNA Polymerase.

Differences between DNAP and RNAP

- No primer is needed in RNAP
- No proofreading activity in RNAP.

Prokaryotic RNA Polymerase

- Only one type[Q] of Prokaryotic RNA Polymerase
- Multisubunit Enzyme
- Core Enzyme + σ subunit = Holoenzyme (E σ)
- Core Enzyme consist 2 α and 1β and1 β' and ω subunit
- σ subunit[Q] help RNA polymerase to bind to the promoter[Q] site
- β subunit[Q] is the catalytic subunit
- β subunit[Q] binds the Mg^{2+} ions.

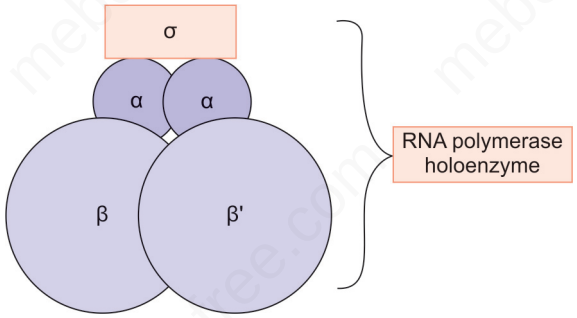


Fig. 11.1: Prokaryotic RNA polymerase

Eukaryotic RNA Polymerases

There are three[Q] types of Eukaryotic RNA Polymerase.

They are more complex than prokaryotic RNA polymerase with a number of subunits.

Form of RNA Polymerase	Sensitivity to α-Amanitin	Major Products of RNAP
RNA Polymerase I	Insensitive	rRNA
RNA Polymerase II	High sensitivity	mRNA, miRNA, SnRNA, lnc RNA
RNA Polymerase III	Intermediate sensitivity	tRNA, 5s rRNA

Promoters of Transcription

Defined as the short conserved sequence in the coding strand of the DNA that specifies start site of the transcription.

Bacterial Promoters are:

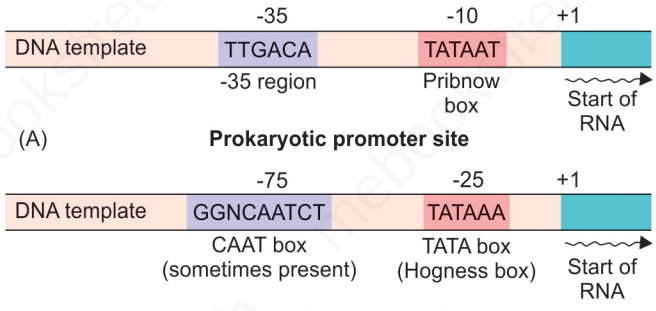
- TATA Box (5′ TATAAT 3′) or Pribnow Box
  10 bp upstream of the start site of transcription (–10 bp)
- TGG Box (5′ TGTTGACA 3′)
  35 bp upstream of the start site of transcription (–35 bp).

Eukaryotic Promoters are:

- Golberg Hogness Box[Q] (similar to TATA Box) (5′ TATAAAG 3′)
  25 to 35 bp upstream of the start site of transcription (–25 to –35 bp)
- CAAT Box
  70 to 80 bp upstream of the start site of transcription
- GC-rich region (GC box)
- Apart from this additional elements like Inr (Initiator sequence) and DPE (Downstream promoter Element) can serve as promoters.

Remember

- Usually promoters are located upstream of the start site of transcription
- But promoters for RNA Polymerase III (transcribe tRNA) are located downstream within the gene
- The promoter sequence (e.g. TATAAT) is on the coding strand of the DNA
- Complementary sequence to the above promoter sequence is seen on the template strand
- σ subunit of RNA Polymerase bind to promoter on the template strand.



Figs 11.2 A and B: Promoters of transcription

Transcription Unit

The region of DNA that includes signals of transcription initiation, elongation and termination.

Enhancers and Repressors

Certain DNA elements facilitate or enhance initiation at the promoter and hence are termed enhancers and those which repress initiation at the promoter are called Repressors or silencers.

Enhancer elements, typically contain multiple binding sites for transactivator proteins.

Properties of Enhancers[Q]

- Can be located upstream or downstream of the transcription site
- Work when located long distances from the promoter
- Work when upstream or downstream from the promoter
- Work when oriented in either direction
- Can work with homologous or heterologous promoters
- Work by binding one or more proteins
- Work by facilitating binding of the basal transcription complex to the cis-linked promoter.

Cis–acting Elements

- Promoters and enhancers that are located on the same DNA are called cis-acting elements.

Trans-acting Elements

- Trans-acting proteins are the products of a separate gene that interacts with the cis-acting elements.

The Transcription Cycle

Transcription can be described in six steps:

- **Template binding and closed RNA polymerase-promoter complex formation:**
  RNA polymerase (RNAP) binds to DNA and then locates a promoter (P) by means of sigma subunit.
- **Open promoter complex formation:**
  Once bound to the promoter, RNAP melts the two DNA strands to form an open promoter complex.
  This complex is also referred to as the pre-initiation complex or PIC.
  Strand separation allows the polymerase to access the coding information in the template strand of DNA.
- **Chain initiation:**
  Using the coding information of the template RNAP catalyzes the coupling of the first base (often a purine) to the second, to form a dinucleotide.
- **Promoter clearance:**
  After RNA chain length reaches ~10–20 nucleotides, the polymerase undergoes a conformational change,

then it moves away from the promoter, transcribing down the transcription unit.

- **Chain elongation:**
  Successive residues are added to the 3′ –OH terminus of the nascent RNA molecule until a transcription termination signal (T) is encountered.
- **Chain termination and RNAP release:**
  By two methods:
  - ρ **(rho) factor dependent termination**Q
    Termination signal for transcription in the template strand is identified by ρ factor.
    ρ factor is an ATP dependent RNA-DNA helicase that disrupts the nascent RNA-DNA complex.
  - **Intrinsic (Spontaneous) or** ρ (rho) factor **independent termination**
    RNA polymerase identifies the termination signal on the template strand without the aid of ρ factor.
    But for this termination the nascent RNA should have certain pre-requisites.
    i. GC rich region that forms a hairpin turn
    ii. U rich region after the GC rich region. The binding of A-U is weak, hence, facilitate termination of transcription.

Similarities between Transcription and Replication

- The general steps of initiation, elongation, and termination with 5′–3′ polarity is present in both.
- Large, multicomponent initiation complexes are involved in both.
- Both obey Watson–Crick base-pairing rules.

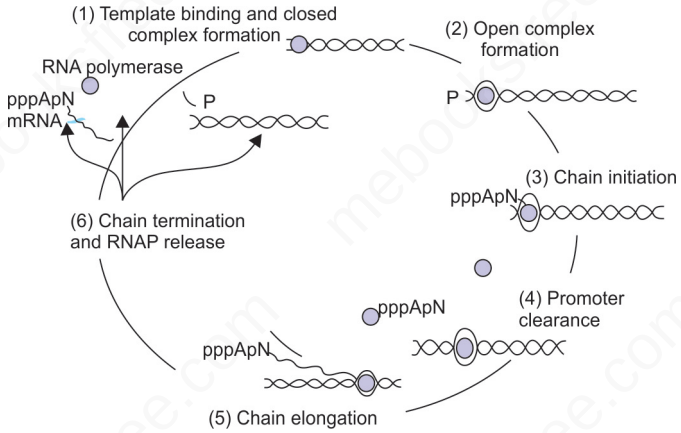


Fig. 11.3: Transcription cycle

Differences between Replication and Transcription

Replication	Transcription
Deoxyribonucleotides are added	Ribonucleotides are added
A is paired with T on the parent strand	U replaces T as the complementary base for A in RNA
Both the strands of DNA act as template	**Only one strand**Q of the DNA acts as the Template
Entire genome must be copied	Only portions of the genome are vigorously transcribed or copied into RNA
A primer is involved as DNA Polymerase cannot initiate DNA Synthesis de novo	**A primer is not involved** as RNA polymerases have the ability to initiate synthesis de novo
Highly active proofreading mechanism	No highly active proofreading mechanism
DNA dependent DNA Polymerase is the enzyme.	DNA dependent RNA Polymerase is the enzyme

Post-Transcriptional Modifications of mRNA or RNA Processing

In Prokaryotes

- mRNA are **not subjected** to post-transcriptional processing.
- Translation is started simultaneous with transcription.
- *Exception*: tRNA and rRNA of prokaryotes undergo post transcriptional modification.

In eukaryotes

- The RNA molecule synthesized by RNA polymerase is known as **Primary Transcript or Heteronuclear RNA (hn RNA)**
- hnRNA undergoes extensive post-transcriptional modification.

Site: The primary transcripts are extensively modified in the nucleus after transcription.

Post-Transcriptional Processing of Primary Transcript of mRNA

- **7-methylguanosine capping at 5′ end**
- **Addition of a poly-A tail at 3′ end**
- Removal of introns and joining of Exons called Splicing
- Methylations
- Alternative RNA Processing

1. **7-methylguanosine capping at 5′ end**
   This takes place in two steps:
   a. Guanosine Triphosphate is attached to the 5′ end of hnRNA
      - By an enzyme Guanylyltransferase

- By an unusual 5′-5′ triphosphate linkage.
- Takes place inside the nucleus.

b. Methylation of Guanosine Triphosphate
- By Guanine 7 methyltransferase.
- S-adenosyl Methionine is the methyl donor.
- Takes place inside the Cytosol.

Functions of 5′ capping

Helps in
- The initiation of translation
- Helps to stabilize the mRNA
- Prevents the attack of 5′ to 3′ exonuclease.

2. **Addition of a poly-A tail at 3′ end**
- Poly A tail is added to the 3′ end of the hnRNA
- Polyadenylate Polymerase is the enzyme
- Takes place in the nucleus
- Length of Poly A tail is up to 200 Adenine bases.

Functions of Poly A tail at the 3′ end.
- Stabilize the mRNA
- Prevents the attack of 3′ to 5′ exonuclease
- Facilitate their exit from the nucleus
- Poly A tail and its binding protein PAB-1 are required for efficient initiation of Protein Synthesis.

3. **Removal of introns and joining of Exons called Splicing**

Intron: Intervening sequence that do not code for amino acid

Exon[Q]: Amino acid coding sequence

Molecular machinery that carry out splicing is called **Spliceosome**.

Spliceosomes

Consist of the primary transcript, five small nuclear RNAs (U1, U2, U4, U5, and U6) and more than 60 protein.

Spliceosome = snRNA + RNP + hnRNA (or mRNA precursor)

Small Nuclear RNA (Sn RNA)
- Uracil rich RNA which can act as enzymes, i.e. Ribozyme.
- U1, U2, U4, U5, U6 involved in mRNA processing.
- U6 is certainly essential, as yeast deficient in this Sn RNA is not viable
- U7 snRNA is involved in production of correct 3' ends of histone mRNA that lacks Poly A tail.

SnRNP complex (Snurps) (Small nuclear ribonucleoprotein)
- SnRNP (Snurps) = Sn RNA + Ribonucleo Protein (RNP)

Clinical Correlation

Systemic lupus erythematosus results from an autoimmune response in which the patient produces antibodies against host proteins, including snRNP (Snurps)

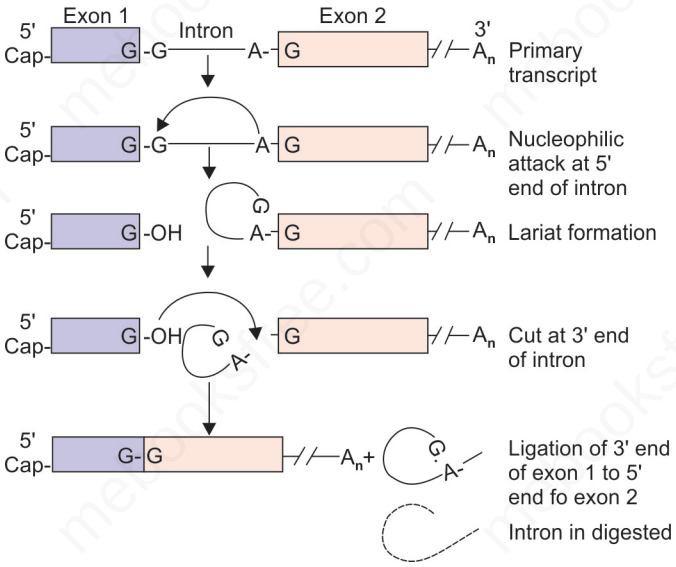


Fig. 11.4: Mechanism of splicing

Mechanism of Splicing

- The binding of snRNP brings the sequences of the neighboring exons into the correct alignment for splicing.
- The splicing start with a cut in the 5′ splice donor site.
- The 2′ –OH group of an adenosine (A) residue (known as the branch site) in the intron attacks the phosphate at the 5′ -end of the intron, forming an unusual 2′ → 5′ phosphodiester bond and creating a **'lariat' structure.**[Q]
- The newly freed 3′ –OH of exon 1 attacks the 5′-phosphate at the splice acceptor site, forming a phosphodiester bond that joins exons 1 and 2.
- The excised intron is released as a lariat, which is degraded.
- After introns have been removed and exons joined, the mature mRNA molecules leave the nucleus and pass into the cytosol through pores in the nuclear membrane.

Self Splicing[Q]

- Certain hnRNA itself has splicing activity
- Because of Ribozyme activity.

Effect of Splice site Mutations

Mutations at splice sites can lead to improper splicing (faulty splicing) and the production of aberrant proteins.

For example, mutations that cause the incorrect splicing of β-globin mRNA are responsible for some cases of β-thalassemia.

4. **Methylations**
   - Methylation of N7 of Adenine and 2′ hydroxyl group of ribose
   - Takes place in the cytoplasm.
5. **Alternative processing of mRNA precursor or Alternative Splicing**
   - A mechanism for producing a diverse set of proteins from a limited set of genes.
   - The pre-mRNA molecules from same genes can be spliced in two or more alternative ways in different tissues.

The mechanisms for alternative processing of mRNA precursors
- Selective Splicing—selective inclusion or exclusion of exons
- Alternative 5' donor site—5' donor site of certain exons is changed.
- Alternative 3' acceptor site—3' acceptor site of certain exons is changed.
- Alternative Polyadenylation site—Different site is used for Poly-adenylation.

Applications of Alternative mRNA Processing

- Generation of membrane bound or Secretory IgG by alternative polyadenylation sites.Q
- Production of several tissue specific isoforms of tropomyosin from single mRNA transcript.

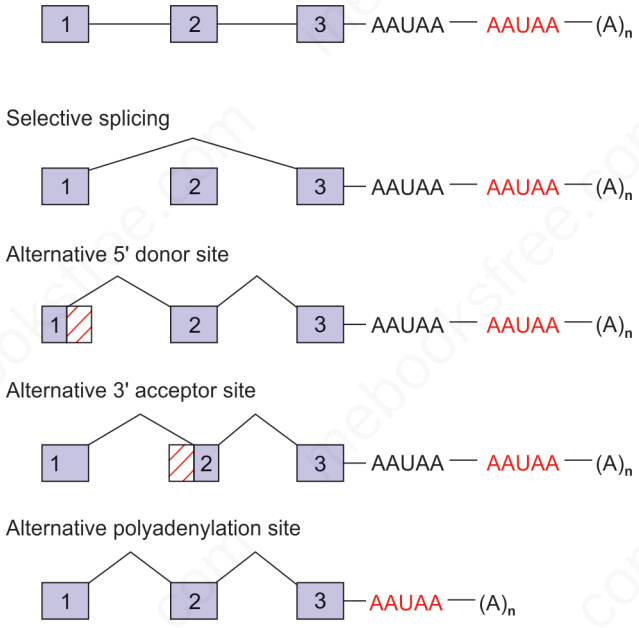


Fig. 11.5: Methods of alternative splicing

RNA Editing

- mRNA editing is an exception to central dogma of molecular genetics
- Current estimate suggest that 0.01% of mRNA is edited in this fashion
- The central dogma states that for a given gene and gene product there is a linear relationship between the coding sequence in DNA, the mRNA sequence, and the protein sequence
- Changes in the DNA sequence should be reflected in a change in the mRNA sequence and, depending on codon usage, in protein sequence
- mRNA editing is the process by which coding information is changed at the mRNA level by chemical modification of the nitrogenous bases present in the codons
- Hence, the linear relationship between the coding sequence in DNA, the mRNA sequence, and the protein sequence is altered
- Thus, it is an exception to Central Dogma of molecular genetics.

An example is the apolipoprotein B (*apoB*) gene and mRNA.

In the liver,

- The single *apoB* gene is transcribed into an mRNA that directs the synthesis of a 512kDa protein, apo B100 with 4536 amino acid residues.

In the intestine,

- The same gene directs the synthesis of the primary transcript
- A **cytidine deaminase** converts a CAA codon in the mRNA to UAA at a single specific site
- Rather than encoding glutamine, this codon becomes a termination signal, and a truncated 242kDa protein (apo B48) with 2512 amino acid residues is the result.

Other examples of RNA editing

- Glutamate Receptor (Glutamine changed to Arginine)
- Trypanosome mitochondrial DNA.

DIFFERENT CLASSES OF RNA

CLASSES OF RNAs

RNA exists in two major classes:

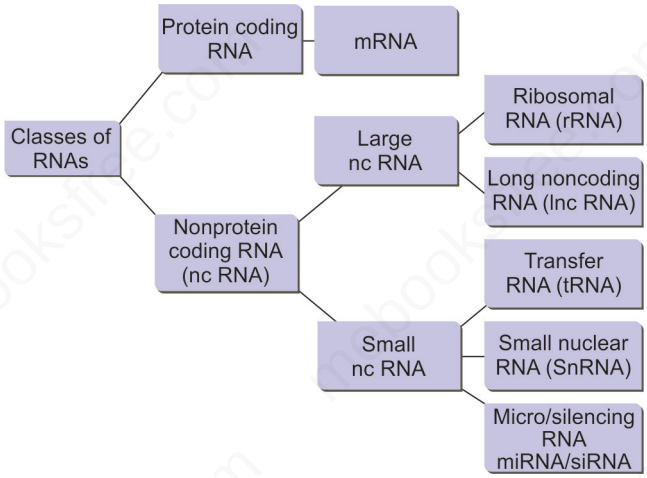


Fig. 11.6: Classes of RNA

RNA	Types	Abundance	Stability
Protein coding RNAs			
Messenger (mRNA)	~10^5 Different species	2–5% of total	Unstable to very stable
Large ncRNAs			
Ribosomal (rRNA)	28S, 18S, 5.8S, 5S	80% of total	Very stable
lnc RNA	~1000s	~1%–2%	Unstable to very stable
Small noncoding RNA (Small ncRNA)			
Transfer (tRNA)	~60 Different species	~15% of total	Very stable
Small nuclear (snRNA)	~30 Different species	1% of total	Very stable
Micro/Silencing (mi/SiRNA)	100s–1000	<1% of total	Stable

Messenger RNA (mRNA)

- Most heterogenous RNA
- Function as messenger conveying information to translation machinery
- 2-5% of total cellular RNA
- 5′ end capped by 7 Methylguanosine triphosphate
- 3′ end nongenetically encoded by polymer of 20–250 adenylate residues.

Ribosomal RNA (rRNA)

- Most abundant RNA is **rRNA** (80% of total RNA)
- Function-Forms Protein Synthesising Machinery called **Ribosome**
- Ribosomal Assembly is ribosomal RNAs associated with certain proteins (i.e. rRNA + Proteins).

Ribosomal Assembly in Prokaryotes[Q]
70S Ribosome = 30S + 50S Subunits
In 30S Subunit 16S rRNA + Proteins
In 50S subunit 23S rRNA+ 5S rRNA + Proteins
Ribosomal Assembly in Eukaryotes[Q]
80S Ribosome = 40S Subunit + 60S Subunit
60S Subunit = 28S rRNA + 5.8S r RNA + 5S rRNA + ~50 proteins
40S =18S rRNA + ~30 proteins

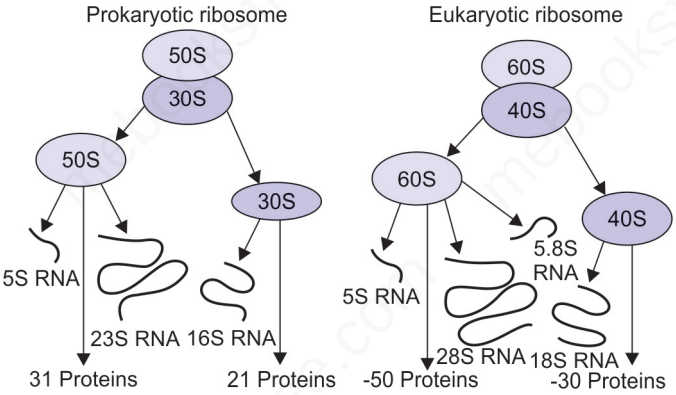


Fig. 11.7: Ribosomal assembly in prokaryotes and eukaryotes

Transfer RNA (tRNA)

- RNA which transfer amino acid from the cytoplasm to the ribosomal protein synthesizing machinery
- **Clover leaf shape** in the secondary structure
- **L shaped** tertiary structure
- Single tRNA contains 74-95 nucleotides
- Cytoplasmic translation system possess 31 tRNA species
- Mitochondrial translation system possess 22 tRNA species.

tRNA contain significant proportion of nucleosides with unusual bases
They are
- DihydroUridine (contain Dihydrouracil)
- Pseudouridine
- Inosine (contain Hypoxanthine)
- Ribothymidine

Arms of tRNA

- **Acceptor arm**
  - Site of attachment of the amino acid
  - 3′ end of the tRNA
  - Has 3 unpaired nucleotide, CCA
  - Carboxyl group of the amino acid is attached to the 3′ hydroxyl group of the adenosyl moiety.

- **Anticodon arm**
  - Has the triplet nucleotide sequence complementary to the codon of the amino acid which the tRNA carries
  - The sequence is read from 3′ to 5′ direction
  - Codon is read from 5′ to 3′ direction
  - Codon of mRNA and anticodon in tRNA are antiparallel in their complementarity.
- **DHU arm**
  - Contain Dihydrouracil residue
  - Acts as the recognition site for specific aminoacyl tRNA synthetase.
- **Pseudouridine arm (TψC arm)**
  - TψC stands for Ribothymidine, Pseudouridine, Cytidine
  - Involved in the binding of aminoacyl tRNA to the Ribosomal surface.
- **Extra arm (Variable arm)**
  - Between Pseudouridine and Anticodon arm
  - The most variable feature of tRNA.
  - Different classes of tRNA is based on the extra arm.

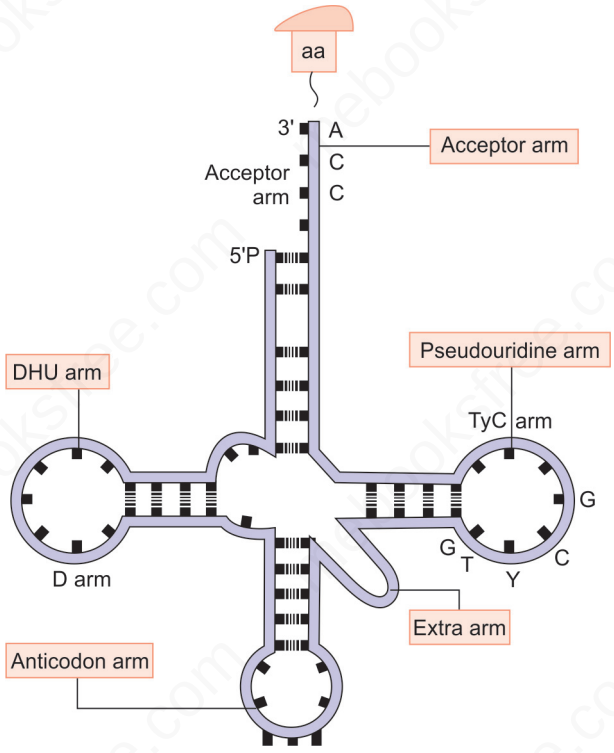


Fig. 11.8: Structure of tRNA

Post-transcriptional modification of tRNA precursor

- Standard bases (A, U, G, C) undergo methylation, reduction, deamination, rearranged glycosidic bond
- Cleavage and attachment of C C A terminal at 3′ end takes place in **cytoplasm**
- Methylation of tRNA takes place in **nucleus**.

Small Nuclear RNA (SnRNA)

- They belong to small RNAs of size 90 to 300 nucleotides
- 1% of total RNAs
- They have ribozyme activity.

Function

- mRNA Processing (U1, U2, U4, 5, 6 and U7 as a part of spliceosome)
- rRNA processing
- Gene regulation.

miRNA and siRNA

- Small **noncoding** single stranded RNAs which are 21-22 nucleotide length
- **Main function[Q]:** Post-transcriptional **regulation of gene expression by targeting mRNA by several distinct mechanism**.

We can discuss the following

- Generation of miRNA and SiRNA
- Post-transcriptional modification of miRNA and siRNA
- Regulation of gene expression by miRNA and siRNA
- Clinical correlations.

Micro RNA (miRNA)

- **Generation of miRNA**
  - Transcribed by RNA Polymerase II from miRNA encoding genes to Pri miRNA.

Post-transcriptional modification of miRNA

Pri miRNA undergo extensine post-transcriptional processing as follows:

- Pri-miRNA is subject to processing by DROSHA-DGCR8 nucleases, which trims 5′ cap and 3′ Poly A tail to generate Pre-miRNA
- The double stranded Pre miRNA is transported to cytoplasm through nuclear pore, Exportin-5
- Pre-miRNA is further trimmed by Dicer nuclease (TRBP-Dicer) to form 21-22 nucleotide miRNA duplex.

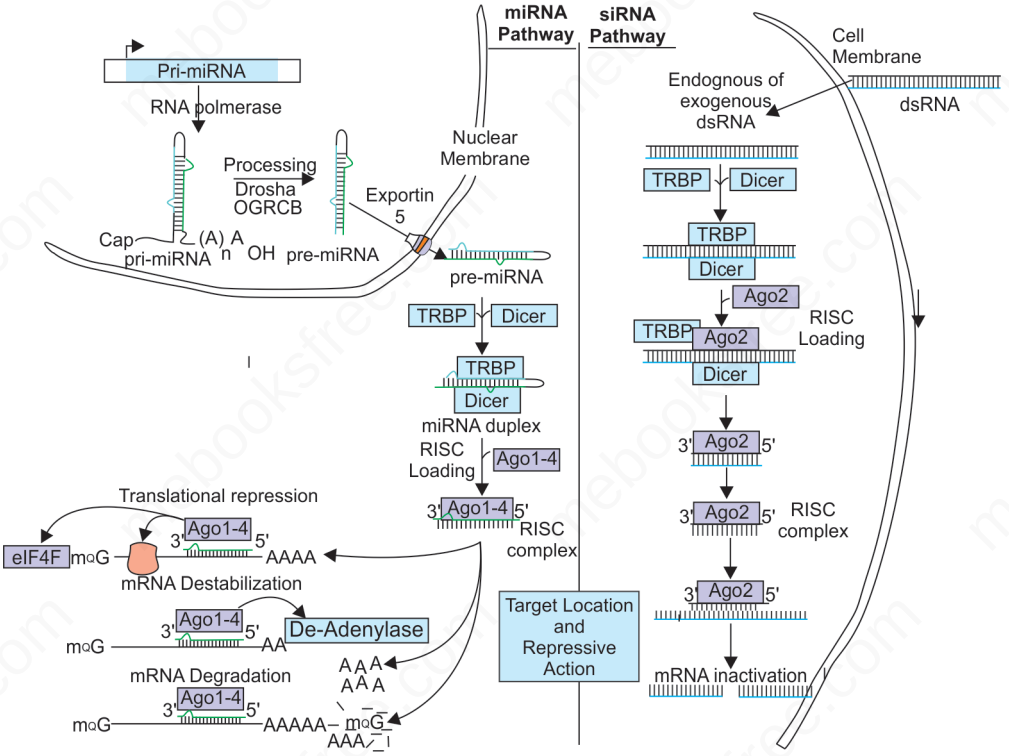


Fig. 11.9: Generation of miRNA and SiRNA

- One of the strand of duplex miRNA is selected.
- The selected strand is loaded to RNA-induced Silencing complex (RISC)
- Mature functional 21–22 nucleotide mi RNA is thus produced.

Silencing RNA or Small Interfering (SiRNA)

Generation of SiRNA

- Functional SiRNA is generated endogenous or exogenous double stranded RNA
- Extracellular sources include RNA Viruses.

Post-transcriptional modification of SiRNA

- Double stranded RNA are processed by Dicer nuclease
- One strand is selected and loaded to RISC.

RISC in miRNA is composed of Argonuate Proteins (Ago 1 → 4)
RISC in SiRNA is composed of Argonuate-2 Proteins

Specific Nucleases Involved in the Post Transcriptional Modification of miRNA and SiRNA

- Drosha-DGCR8^{Q} Nucleases
- Dicer Nucleases

- **The Regulation Gene Expression by mi RNA and si RNA**
  - **By altering mRNA function.**

Regulation of gene expression by miRNA

- Binding of miRNA to mRNA by normal base pairing
- All mRNAs contain a *seed sequence* in their 3′untranslated region (UTR) that determines the specificity of miRNA binding and gene silencing
- If miRNA-mRNA base pairing has one or more mis-matches. Translation of cognate mRNA is inhibited
- If miRNA-mRNA base pairing is perfect, corresponding mRNA is degraded.

miRNA modulate the function of target mRNA by three methods

- Translation repression by targeting 5′ methyl cap binding factor, eIF4 or ribosome directly
- mRNA destabilzation by mRNA Poly A tail deadenylation
- Promoting mRNA degradation directly.

Regulation of gene expression by SiRNA

- Induces mRNA cleavage which inactivate target mRNA.

RNA Interference or RNAi

- Functional Consequence of Translation Arrest and mRNA degradation by miRNA/SiRNA is **Silencing the gene expression or Gene Silencing**

- This is otherwise called **RNA Interference**
- RNAi by miRNA/SiRNA is an example of gene knock down.

P Bodies

- Nontranslating mRNA form ribonucleoprotein particles and they accumulate in cytoplasmic organelle called P bodies
- Ribonucleoprotein or mRNP are mRNA, bound by specific packaging Proteins
- P bodies are sites of translation repression and mRNA decay
- P bodies contain mRNA decapping enzymes, RNA helicases, RNA exonucleases, etc. for mRNA quality control
- A portion of miRNA driven mRNA modulation takes place in P bodies.

In 2006, Craig Mello and Andrew Fire were awarded Nobel Prize for silencing gene expression by mi RNA.

miRNA and Cancer

- Can prevent cancer by degrading mRNA of an oncogene. They are called Tumor Suppressive miRs
- Can cause cancer degrading m RNA of a tumour suppressor gene called oncogenic mRNA (oncomir)

miRNA associated with cancer

Oncomirs

- Mir-21 is one of the widely studies oncogenic miRNA.
- miR-200 promote epithelial-mesenchymal transitions which is important in invasiveness and metastasis of tumor.
- miR-155 is overexpressed in many human B cell lymphomas and indirectly upregulates genes like *MYC*.

Tumor suppressive miRs

- Deletions affecting certain tumor suppressive miRs, such as miR-15 and miR-16, are among the most frequent genetic lesions in chronic lymphocytic leukemia
- Rare ovarian and testicular tumors, associated with germline defects in *DICER*, a gene that encodes an endonuclease

miRNA in DNA repair

- mir-34
- p53, the molecular policeman activate the expression of mir-34.

Application of miRNA/SiRNA

SiRNA/miRNA and Transgenic mice

- Synthetic SiRNA targeted against specific mRNA can be experimentally introduced into cell to study gene function by Gene knock down technology.
- SiRNA can be used as possible therapeutic agents to silence pathogenic genes, such as oncogenes involved in neoplastic transformation.

Long Noncoding RNA (lnc RNA)

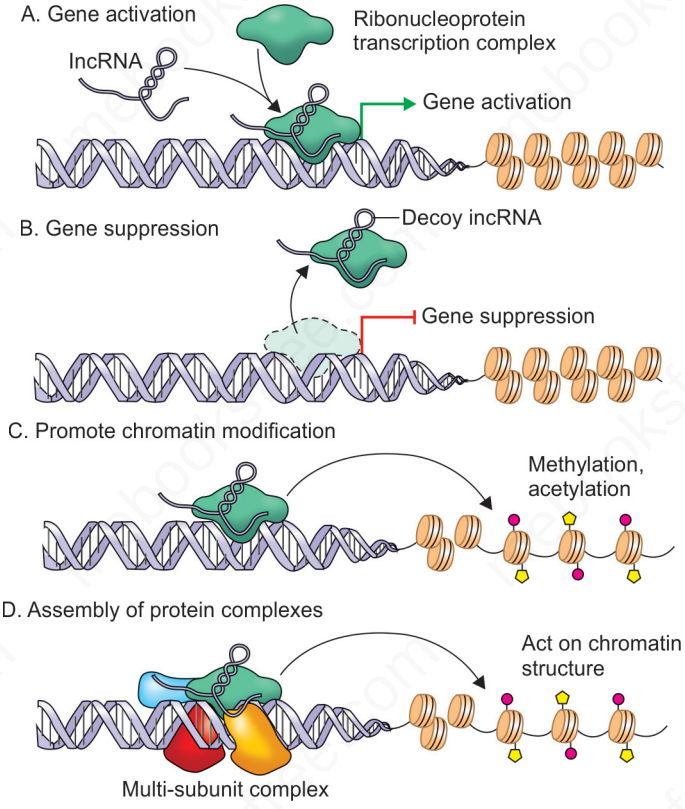
- Transcribed by RNA Polymerase II
- They are >200 nucleotide length
- They modulate gene expression in many ways.

Regulation of gene expression by lnc RNA

- Facilitate transcription factor binding and thus promote gene activation
- Bind to transcription factors and thus prevent gene transcription, e.g. Decoy lnc RNA
  - The best known example of a repressive function involves XIST, which is transcribed from the X Chromosome and plays an essential role in physiologic X chromosome inactivation
- Facilitate Histone and DNA modification by directing methylases or acetylases
- Act as scaffolding and stabilize secondary or tertiary structures and multisubunit complexes that influence chromatin structure.

Some recent facts about lncRNA

- lncRNAs may exceed coding mRNAs by 10- to 20-fold
- It has been recently appreciated that many enhancers are sites of lncRNA synthesis, often increase transcription
- Emerging studies are exploring the roles of lncRNAs in various human diseases, from atherosclerosis to cancer.



Figs 11.10A to D: Regulation of gene expression by lncRNA

hnRNA (Heteronuclear RNA)

- Primary transcript mRNA formed from DNA template
- It underges post transcriptional modification to form mature mRNA.

Newly Described Noncoding RNAs

Small Nucleolar RNA (SnoRNA)

- RNA involved in eukaryotic rRNA Processing and assembly of ribosomes
- Present in the nucleolus.

piwi-interacting RNAs (piRNAs),

- The most common type of small noncoding RNA
- Function: They have a role in post-transcriptional gene silencing (like miRNAs).

Long intervening noncoding RNAs (linc RNAs)

- Function: Regulate the factors that modify histones (epigenetic modifications) and thereby control gene expression.

REVIEW QUESTIONS

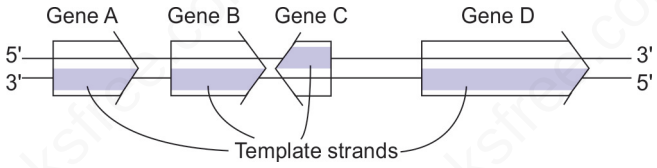
TRANSCRIPTION

1. True about coding strand of DNA: (PGI Dec 03)

a. Template strand
b. Minus strand
c. Runs at 5′–3′ direction
d. Runs at 3′–5′ direction
e. Plus strand

Ans. c, d, e. Runs in 5′–3′ direction, Runs at 3′–5′ direction, Plus strand. *(Harper 30/e page 395)*

- Template strand is in 3′ to 5′ direction, or 5′ to 3′ direction, Minus strand, Antisense strand
- Coding strand is in 5′ to 3′ direction, or 3′ to 5′ direction, Plus strand, Sense strand
- Whatever is the direction of template strand, for transcription, that strand is read always in 3′ to 5′ direction.



This figure from Harper clearly shows template or coding strand can be either 5′ to 3, or 3′ to 5′. But the direction of transcription is 5′ to 3′. The template strand is read always in 3′ to 5′ direction.

2. 5′ TTACGTAC 3′ after transcription what will be the RNA: (PGI June 08)

a. 5′-TTACGTAC 3′
b. 3′-TTACGTAC 5′
c. 5′-CATGCATT 3′
d. 3′-CATGCATT 5′
e. 5′-GUACGUAA 3′

Ans. e. 5′-GUACGUAA 3′.

- Here question is to find the RNA, so all options with Thymine is not the answer. So answer is E
- The exact method is to find the complementary sequence for the given DNA and at the places of T, replaced by U
- 5′ TTACGTAC 3′ is read in 3′ to 5′ direction
- So RNA is 5′ GUACGUAA 3′.

3. Immunoglobulin molecule is synthesized by in mixed or separate due to: (AIIMS May 2012)

a. Co-dominance
b. Gene switching
c. Allele exclusion
d. Differential RNA processing

Ans. d. Differential RNA processing.

(Ref: Harper 30/e page 449)

Alternative RNA Processing, which includes Alternative Polyadenylation site in the μIg heavy chain primary transcript, results in two μ protein, μ_m and μ_s. One remains membrane bound to B lymphocyte (μ_m) and the other secreted (μ_s).

4. A four-year-old child is diagnosed with Duchenne muscular dystrophy, an X-linked recessive disorder, Genetic analysis shows that the patient's gene for the muscle protein dystrophin contains a mutation in its promoter region. What would be the most likely effect of this mutation? (Nov 2010)

a. Tailing of dystrophin mRNA will be defective
b. Capping of dystrophin mRNA will be defective
c. Termination of dystrophin transcription will be deficient

d. Initiation of dystrophin transcription will be deficient

Ans. d. Initiation of dystrophin transcription will be deficient. *(Ref: Harper 30/e page 397-400)*

The fidelity and frequency of transcription is controlled by proteins bound to certain DNA sequences. These regions are termed promoters, and it is the association of RNAP with promoters that ensures accurate initiation of transcription.

Promoters are responsible for initiation process of transcription.

5. Splicing activity is a function of: **(AIIMS Nov 2010)**

a. mRNA
b. snRNA
c. tRNA
d. rRNA

Ans. b. sn RNA. *(Harper 30/e page 367,411)*

Small Nuclear RNA (Sn RNA)

Uracil rich RNA which can act as enzymes, i.e. Ribozyme.

U1, U2, U4, U5, U6 involved in mRNA processing.

U7 in processing histone mRNA.

6. Post-transcriptional modification includes: **(PGI Nov 2012)**

a. All RNA undergo post-transcriptional modification
b. Capping of pre mRNA involves the addition of 7-methylguanosine to the 5′ end
c. Poly A tail occur at 3′ end
d. Intron excision by spliceosome
e. Primarily occurs in the cytoplasm

Ans. a, b, c, d. All RNA undergo post ....., Capping of pre mRNA ....., Poly A tail occur at 3′ end, Intron excision by spliceosome.

Post-Transcriptional Processing Includes:

Primarily occurs in the nucleus

7-methylguanosine Capping at 5′ End

This takes place in two steps.

- Guanosine Triphosphate is attached to the 5′ end of hnRNA
  - By an enzyme Guanylyltransferase
  - By an unusual 5′-5′ triphosphate linkage
  - Takes place inside the **nucleus.**
- Methylation of Guanosine Triphosphate
  - By Guanine 7 methyltransferase
  - S-adenosyl Methionine is the methyl donor
  - Takes place inside the Cytosol.

Addition of a Poly-A Tail at 3′ End

- Poly A tail is added to the 3′ end of the hnRNA
- Polyadenylate Polymerase is the enzyme
- Takes place in the **nucleus**
- Length of Poly A tail is up to 200.

Removal of Introns and Joining of Exons called Splicing

- Intron-Intervening Sequence that does not code for amino acid
- Exon-Amino Acid Coding Sequence
- Molecular machinery that carry out splicing is called Spliceosome.

7. Noncoding RNAs are: **(PGI May 2012)**

a. siRNA
b. miRNA
c. tRNA
d. mRNA
e. rRNA

Ans. a, b, c, e. *(Harper 30/e page 395)*

Noncoding RNAs (ncRNA)

- Transfer RNA (tRNA)
- Ribosomal RNA (rRNA)
- Sno RNA
- gRNA (Guide RNA)
- miRNA
- siRNA
- lncRNA.

8. Not a product of transcription **(PGI May 2011)**

a. tRNA
b. mRNA
c. rRNA
d. cDNA
e. New strand of DNA

Ans. d, e. cDNA, New strand of DNA.

(Ref: Harper 30/e page 395-398)

cDNA is produced by reverse transcription.

New DNA strand produced by DNA replication.

Form of RNA Polymerase	Sensitivity to α-Amanitin	Major Products of transcription
RNA Polymerase I	Insensitive	rRNA
RNA Polymerase II	High sensitivity	mRNA, miRNA, SnRNA, lnc RNA
RNA Polymerase III	Intermediate sensitivity	tRNA, 5s rRNA

9. Reverse transcriptase is: (PGI May 2011)

a. DNA dependent RNA polymerase
b. RNA dependent DNA polymerase
c. DNA dependent DNA polymerase
d. RNA dependent RNA polymerase
e. RNA polymerase

Ans. b. RNA dependent DNA polymerase.

(Ref: Harper 30/e page 363)

Reverse Transcription

- RNA converted to DNA is reverse transcription
- The enzyme is called Reverse Transcriptase
- This is otherwise RNA dependent DNA Polymerase.

Remember

- DNA Polymerase is DNA dependent DNA Polymerase
- Reverse Transcriptase is RNA dependent DNA Polymerase
- Primase is DNA Dependent RNA Polymerase.

10. Which type of RNA has the highest percentage of modified base? (AI 2006)

a. mRNA
b. tRNA
c. rRNA
d. snRNA

Ans. b. tRNA. *(Ref: Harper 29/e page 411)*

Transfer RNA (tRNA)

- RNA which transfer amino acid from the cytoplasm to the ribosomal protein synthesizing machinery
- Clover leaf shape in the secondary structure
- L-shaped tertiary structure
- Single tRNA contains 74-95 nucleotides
- Cytoplasmic translation system possess 31 tRNA species
- Mitochondrial system possess 22 tRNAs.

Contain significant proportion of nucleosides with unusual bases.

They are:

- Dihydro uridine (contain Dihydrouracil)
- Pseudouridine
- Inosine (contain Hypoxanthine)
- Ribothymidine.

11. The sigma (s) submit of prokaryotic RNA polymerase: (AI 2006)

a. Binds the antibiotic rifampicin
b. Is inhibited by a-amanitin
c. Specifically recognizes the promoter site
d. Is part of the core enzyme

Ans. c. Specifically recognizes the promoter site.

(Ref: Harper 30/e page 399)

Prokaryotic RNA Polymerase

Only one type of Prokaryotic RNA Polymerase

Multisubunit Enzyme

- Core Enzyme + σ subunit = Holoenzyme (E σ)
- Core Enzyme consist 2 α and 1β and1 β′ and ω subunit
- σ subunit help RNA polymerase to bind to the promoter site
- β subunit is the catalytic subunit
- β subunit binds the Mg^{2+} ions.

12. The base sequence of the strand of DNA used as a template has the sequence 5′ GATCTAC 3′. What would be the base sequence of RNA product? (Ker 2012)

a. 5′ CTAGATG 3′
b. 5′ GAUCUAC 3′
c. 5′ GTAGATC 3′
d. 5′ GUAGAUC 3′

Ans. d. 5′ GUAGAUC 3′

Read the strand in 3′ to 5′ direction. Write the complementary sequence in 5′ to 3′ direction obeying base pairing rule, except in the case of T replaced by U.

13. Most common RNA is: (Ker 2011)

a. rRNA
b. mRNA
c. tRNA
d. hnRNA

Ans. a. rRNA. *(Harper 30/e page 395)*

RNA	Types	Abundance	Stability
Ribosomal (rRNA)	28S, 18S, 5.8S, 5S	80% of total	Very stable
Messenger (mRNA)	~10^5 Different species	2–5% of total	Unstable to very stable
Transfer (tRNA)	~60 Different species	~15% of total	Very stable
Small RNAs			
Small nuclear (snRNA)	~30 Different species	1% of total	Very stable
Micro (miRNA)	100s–1000	<1% of total	Stable

14. DNA dependent RNA polymerase is seen in: (Ker 2008)

a. Primase
b. DNA polymerase I
c. DNA polymerase III
d. DNA gyrase

Ans. a. Primase. *(Ref: Harper 29/e page 366)*

Primase enzyme synthesize RNA primer on the DNA strand, hence, it is DNA dependent RNA Polymerase.

15. Strand of DNA from which mRNA is formed by transcription is called: (Ker 2006)

a. Template
b. Anti-template
c. Coding
d. Transcript

Ans. a. Template. *(Ref: Harper 30e page 395)*

16. On which of the following tRNA acts specifically.

a. ATP
b. Golgi body
c. Specific amino acid
d. Ribosome

Ans. c. Specific amino acid. *(Harper 29/e page 397)*

At Least one Species of Transfer RNA (tRNA) Exists for Each of the 20 Amino Acids

- tRNA molecules have extraordinarily similar functions and three-dimensional structures. The adapter function of the tRNA molecules requires the charging of each specific tRNA with its specific amino acid.

17. In conversion of DNA to RNA, enzyme required: (PGI June 08)

a. DNA-polymerase
b. DNA Ligase
c. DNA polymerase III
d. RNA polymerase
e. Primase

Ans. d. RNA Polymerase. *(Harper 30/e page 395)*

Primase, DNA Polymerase & DNA Ligase for replication.

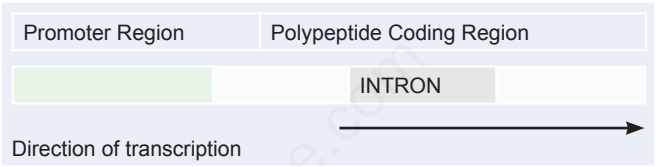
18. RNA polymerase does not require: (AI 2004)

a. Template (ds DNA)
b. Activated precursors (ATP, GTP, UTP, CTP)
c. Divalent metal ions (Mn^{2+}, Mg^{2+})
d. Primer

Ans. d. Primer. *(Harper 30/e page 395, 397)*

- RNA Polymerase does not require Primer.

The following is a generalized diagram of typical eukaryotic gene: *(AIIMS May 06)*



19. What is the most likely effect of a 2 bp insertion in the middle of the intron?

a. Normal transcription, altered translation
b. Defective termination of transcription, normal translation
c. Normal transcription, defective mRNA splicing
d. Normal transcription, Normal translation

Ans. d. Normal transcription, Normal translation.

- As the insertion is in the middle of the noncoding region, intron
- Transcription is normal
- As the intron is spliced out and only exons join together, translation is also normal.

20. In a DNA, the coding region reads 5′-CGT-3′. This would code in the RNA as: (AIIMS May 03)

a. 5′-CGU-3′
b. 5′-GCA-3′
c. 5′-ACG-3′
d. 5′-UGC-3′

Ans. a. 5′-CGU-3′ *(Harper 30/e page 395)*

As the question specifies that it is coding region, so the RNA product is same as coding region except in the case of T replaced by U.

21. Cytoplasmic process during processing is: **(SGPGI 05, CMC 03)**

a. 5′ capping
b. Poly A tailing
c. Methylation of tRNA
d. Attachment of CCA in tRNA

Ans. d. Attachment of CCA in tRNA.

(Harper 30/e page 411)

Sites of post-transcriptional processing

tRNA

- Cleavage and attatchment of CCA terminal at 3′ end takes place in cytoplasm
- Methylation of tRNA takes place in nucleus.

mRNA

- Poly A tail in Nucleus
- 5′ MeGTP capping in nucleus and cytoplasm
- Methylation of some residues in Cytoplasm.

rRNA

- Most common site is nucleolus.

22. All are the processing reaction in tRNA, except: **(WB 03, Delhi 04, UP 05)**

a. CCA tailing
b. Methylation of bases
c. Poly A tailing
d. Trimming of 5′ end

Ans. c. Poly A tailing.

Poly A tailing is a processing step of mRNA primary transcript.

23. Introns are exised by: **(PGI Dec 05)**

a. RNA splicing
b. RNA editing
c. Restriction endonuclease
d. DNAase
e. Helicase

Ans. a. RNA splicing

Removal of introns and joining of Exons called Splicing

Intron: Intervening sequence that do not code for amino acid.

Exon^{Q}-Amino acid coding sequence.

Molecular machinery that carries out splicing is called **Spliceosome**.

Spliceosomes

Consist of the primary transcript, five small nuclear RNAs (U1, U2, U4, U5, and U6) and more than 60 protein. Spliceosome = snRNA + RNP + hnRNA (or mRNA precursor).

24. A segment of eukaryotic gene that is not represented in the mature mRNA is known as: **(AI 2004)**

a. Intron
b. Exon
c. Plasmid
d. TATA box

Ans. a. Intron. *(Harper 30/e page 409)*

25. An enzyme that makes a double stranded DNA copy from a single stranded RNA template molecule is known as: **(AI 2004)**

a. DNA polymerase
b. RNA polymerase
c. Reverse transcriptase
d. Phosphokinase

Ans. c. Reverse transcriptase.

26. Thymidylated RNA present in: **(PGI June 01)**

a. mRNA
b. rRNA
c. tRNA
d. 16-s-RNA

Ans. c. tRNA. *(Harper 30/e page 409)*

Pseudouridine Arm (TψC arm) of tRNA

TψC stands for Ribothymidine, Pseudouridine, Cytidine.

27. Function of Pseudouridine arm of tRNA **(JIPMER 2015 Nov)**

a. Helps in initiation of translation
b. Serves as the recognition site of amino acyl tRNA synthetase
c. Recognises the triple nucleotide codon present in the mRNA
d. Helps in initiation of transcription

Ans. a. Helps in initiation of translation.

(Harper 30/e page 409)

Arms of tRNA

1. **Acceptor Arm**
   Site of attachment of the Amino Acid
   3′ end of the tRNA

Has 3 unpaired nucleotide, CCA

Carboxyl group of the amino acid is attached to the 3′ hydroxyl group of the adenosyl moiety.

2. **Anticodon arm**

Has the triplet nucleotide sequence complementary to the codon of the amino acid which the tRNA carries.

The sequence is read from *3′ to 5′ direction*

Codon is read from 5′ to 3′ direction

Codon of mRNA and anticodon in tRNA are antiparallel in their complementarity.

3. **DHU Arm**

Contain Dihydrouracil residue.

Acts as the recognition site for specific aminoacyl tRNA synthetase.

4. **Pseudouridine Arm (TψC arm)**

- TψC stands for Ribothymidine, Pseudouridine, Cytidine
- Involved in the binding of aminoacyl tRNA to the Ribosomal surface. This helps in the formation of initiation complex.

28. True about 3′ exonuclease: (PGI May 2014)

a. Cleave 3′ end of DNA
b. Cleave 5′ end of DNA
c. Cleave 3′ end of RNA
d. Cleave 5′ end of RNA

Ans. a. Cleaves 3′ end of DNA.

29. Which is a reverse transcriptase: (Jipmer 2014)

a. Topoisomerase
b. Telomerase
c. RNA polymerase II
d. DNA polymerase alpha

Ans. b. Telomerase.

Reverse transcriptase is RNA dependent DNA Polymerase:

- Option a: Topoisomerase is a nicking resealing enzyme, not a reverse transcriptase
- Option b: Telomerase has reverse transcriptase activity
- Option c: RNA polymerase II is an DNA dependent RNA Polymerase, not a reverse transcriptase
- Option d: DNAP alpha is a eukaryotic DNA dependent DNA Polymerase. But also has Primase activity

  Primase is DNA dependent RNA Polymerase, not a reverse transcriptase.

30. Which of the following is true regarding transcription except: (PGI)

a. mRNA formed
b. DNA polymerase enzyme is used
c. RNA polymerase enzyme is used
d. Eukaryotes possess 3 different types of RNA polymerase

Ans. b. DNA Polymerase enzyme is used.

(Harper 30/e page 395-397)

- DNA Polymerase is the enzyme of replication.

31. Apo B48 and Apo B100 is synthesized from the mRNA; the difference between them is due to: (AIIMS May 2011)

a. RNA splicing
b. Allelic exclusion
c. Deamination of cytidine to uridine
d. Upstream repression

Ans. c. Deamination of Cytidine to Uracil.

(Ref: Harper 30/e page 393)

mRNA editing

- The central dogma states that for a given gene and gene product there is a linear relationship between the coding sequence in DNA, the mRNA sequence, and the protein sequence
- Changes in the DNA sequence should be reflected in a change in the mRNA sequence and, depending on codon usage, in protein sequence
- mRNA editing is the process by which coding information is changed at the mRNA level by chemical modification of the nitrogenous bases present in the codons
- Hence, the linear relationship between the coding sequence in DNA, the mRNA sequence, and the protein sequence is altered
- Thus, it is an exception to Central Dogma of molecular genetics.

An example is the apolipoprotein B (apoB) gene and mRNA.

- In the liver, the single apoB gene is transcribed into an mRNA that directs the synthesis of a 100-kDa protein, apoB100
- In the intestine, the same gene directs the synthesis of the primary transcript
- A cytidinedeaminase converts a CAA codon in the mRNA to UAA at a single specific site
- Rather than encoding glutamine, this codon becomes a termination signal, and a 48-kDa protein (apoB48) is the result.

12 Translation

Topics Included

- Translation
- Codon and Genetic Code
- Inhibitors of Protein Synthesis

TRANSLATION

Definition

- The process by which message in the genetic code in the mRNA is translated into sequence of amino acids in the proteins.

Codon

The triplet nucleotide sequence present in the mRNA representing specific amino acid.

- If 1 base represent 1 amino acid only 4 amino acids
- If 2 base represent 1 amino acid 4^2 Aminoacidsie 16 Amino acids
- If 3 base 4^3, i.e. 64 amino acids
- If 4 bases 4^4, i.e. 256 amino acids[Q]

tRNA as an Adapter Molecule

- The language of nucleotide is translated to language of amino acid in translation
- The codon has no affinity towards the amino acid that it codes
- tRNA acts as the adapter molecule between the codon and the specific amino acid
- This is possible by two reasons
- Nucleotide sequence of codon is complementary to the anticodon in the tRNA
- DHU arm recognize the specific Amino acyl tRNA synthetase, so that specific amino acid bind to the Acceptor arm of the tRNA.

For example:

- UUU is the codon for Phenylalanine
- The tRNA that carries Phenylalanine has GGG in the anticodon arm
- DHU arm recognizes the Phenylalanyl tRNA Synthetase.

Genetic Code

- The whole set of codons representing all the amino acids is called Genetic Code
- Cracking of Genetic Code was done by **Marshal Nirenberg and Har Gobind Khorana**.

Salient Features of Genetic Code

- **Triplet Codon:** Each amino acid is represented by triplet sequence
- **Degenerate (Redundant)**[Q]
  - More than 1 codon represent a single amino acid
  - Degeneracy of the codon lies in the **3rd Base**.[Q]

Two amino acids with single codon

  - AUG --- Methionine
  - UGG ---- Tryptophan
- **Nonoverlapping**
  Reading of genetic code does not involve overlapping sequence.

Amino acids with maximum number of codons (Six codons)

- Serine, Arginine, Leucine

- **Unambiguous**
  - Any specific codon can represent only one amino acid.
- **Universal**
  - A specific codon represent a specific amino acid in all the species
  - Exception to this rule–Codons of Mitochondrial DNA.

- **Initiator codon**
  - In eukaryotes ---- AUG codes for Met
  - In Prokaryotes ---- AUG codes for N-Formyl Methionine
- **Terminator Codons**
  - UAG ---- Amber
  - UGA ---- Opal
  - UAA ---- Ochre

Exceptions:
- UGA can be recoded to Selenocysteine
- UAG can be recoded to Pyrrolysine
- UGA codes for Tryptophan in mitochondrial DNA.

Wobbling Phenomenon

The base pairing at the 3rd nucleotide between the anticodon in the tRNA and Codon in the mRNA is not stringently regulated. This is called Wobbling phenomenon.

For example:
- Two codons for Arginine are AGA and AGG can bind with same tRNA having UCU in the anticodon arm
- Base pairing at the third nucleotide is not always obeying base-pairing rule
- Thus, it is said degeneracy lies in the third base
- This explains how 31 tRNA species can bind with 61 coding codons.

Cistron[Q]

It is the smallest unit of genetic expression which code for a polypeptide chain. Monocistronic–one cistron for one Polypeptide

e.g. Eukaryotic mRNA

Polycistronic—One cistron represents more than one polypeptide

e.g. Prokaryotic mRNA
- *Mnemonic: P for P (Prokaryotic is Polycistronic).*

Polarity of Transcription
- The message in the mRNA is decoded from 5' end to 3' end
- The codon in the 5' end of mRNA corresponds to N Terminal amino acid of the polypeptide.

Steps of Protein Synthesis

1. Charging of tRNA
2. Initiation
3. Elongation
4. Termination

1. Charging of tRNA

- The process by which specific amino acid is attached to the acceptor arm by specific aminoacyl tRNA synthetase
- **Specific aminoacyl tRNA Synthetase** enzyme is identified by DHU arm
- The charging reaction has an error rate of less than 10^{-4}
- Hence, **aminoacyl tRNA Synthetase is considered as the proofreading mechanism of translation**
- 2 inorganic Phosphates are used in the charging of the tRNA.

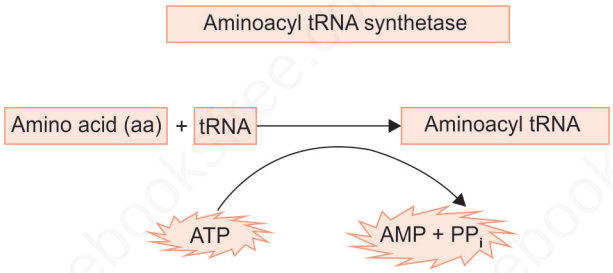


Fig. 12.1: Charging of tRNA

2. Initiation

Identification of initiator codon.

By Marker Sequence—Consensus sequence that helps in the identification of initiator codon.
- Prokaryotes: **Shine-Dalgarno sequence**
- Eukaryotes: **Kozak sequence.**

The first AUG sequence after the marker sequence is defined as the start codon.

AUG codon binds with met tRNA[1].

Initiation
- Is a multistep process
- Facilitated by accessory proteins called Initiation factors
- In case of eukaryotes it is called eukaryotic Initiation Factors (eIF)

1. **Dissociation of the Ribosome into its 40S and 60S Subunits**
   - Two initiation factors delays the association of 40S Subunit and 60S Subunit
   - They are eukaryotic Initiation Factor -3 (eIF-3) and eIF-1A.
2. **Formation of 43S Pre-initiation Complex**

   Three steps:
   - First step involves binding of GTP by eIF-2 to form **Binary Complex**
   - Second step involves binding of binary complex to met tRNAi to form **Ternary Complex**
   - This ternary complex binds to 40S Subunit of Ribosome to form **43S Pre-initiation complex**.

In Stressful conditions kinases are activated.
eIF2α is phosphorylated and protein synthesis is arrested during stressful conditions.
This explains how protein synthesis is decreased in glucose starvation, viral infection etc.

3. **Formation of 48S Initiation Complex**
   - Binding of 43S Pre-initiation complex to the mRNA forms 48S Initiation Complex
   - Formation of 48S Initiation Complex *require ATP Hydrolysis*.

Factors that facilitate the binding of mRNA to 40S Subunit

- 5′ methyl Guanosine cap and Cap binding Complex (Described below)
- 3′ Poly A tail and Poly A tail Binding Protein (PAB-1).

Cap Binding Complex
eIF 4F Complex
- Consist of eIF-4E and eIF4G - eIF4A
- This cap binding complex bind to 7meG cap though **4E**
- This complex is very important in controlling the rate of translation
- 4E responsible for recognition of mRNA cap structure, is a rate limiting step of translation.

Insulin and other mitogenic growth factors through AKT/PI3 Kinase pathway.
This phosphorylates 4E binding protein, this makes 4E free.
This facilitates 4F complex to bind mRNA cap through 4E.
Thus, Insulin increases the rate of initiation of translation.

4. **Formation of 80S Initiation Complex**

48S Initiation Complex + 60S subunit of the ribosome form 80S Initiation Complex.

Involves GTP Hydrolysis.

Three sites are present in the 80S Ribosome:

i. A site-where the new Aminoacyl tRNA binds.
ii. P site-where the growing peptidyl chain present.
iii. E site-where the deacylated tRNA is present.

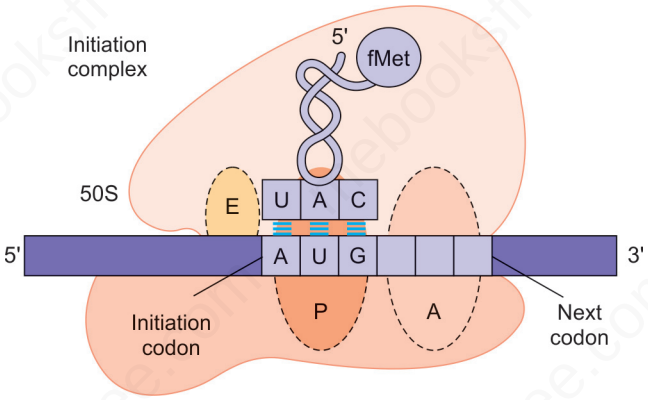


Fig. 12.2: Initiation complex

3. Elongation of Polypeptide in Translation

Multistep Process

Catalyzed by proteins called **Elongation Factors**.

It involves 4 steps:

i. Binding of Aminoacyl tRNA to the A site.
ii. Peptide Bond Formation.
iii. Translocation of ribosome on the mRNA.
iv. Expulsion of deacylated tRNA from P and E site.

Binding of Aminoacyl tRNA to the A site

- tRNA carrying the specific amino acid binds to the A site
- Elongation factor EF-1 helps in the binding of $tRNA^{aa}$
- GTP is hydrolyzed to GDP.

Peptide Bond Formation

- The alpha amino group of the incoming amino acid in the A site forms peptide bond with the COOH group of the peptidyl tRNA in the P (Peptidyl or Polypeptide) site
- Enzyme is Peptidyltransferase, a ribozyme which is a component of *28Sr RNA of 60S Subunit*
- *NO ENERGY IS REQUIRED FOR THIS PEPTIDE BOND FORMATION STEP*
- The growing peptide chain is now in the A site.

Translocation of the ribosome on the mRNA

- The ribosome move forwards, then whole mRNA is shifted by a distance of one codon
- Peptidyl tRNA is translocated to P site from A site
- A site is free to receive the next incoming $tRNA^{aa}$
- Deacylated tRNA is on E site
- Translocation requires EF-2 and GTP.

Termination of Protein Synthesis

- Releasing factor helps in the termination
- Stop codon is in the A site now
- Releasing factor-1 (RF-1) recognises the stop codon in the A site
- RF-1 is bound by RF-3 and GTP
- This complex with peptidyl transferase promote hydrolysis bond between polypeptide chain and tRNA
- This involves hydrolysis of GTP to GDP.

Eukaryotic Initiation Factors (eIF)

eIF-3 and eIF-1A	Bind to newly dissociated 40S ribosomal subunit. This allows translation initiation factor to associate with the 40S subunit
eIF-2	Involved in the formation of binary complex, of 43S preinitiation complex
eIF-4F	Cap binding complex Important in 48S Initiation complex
eIF-5	Hydrolysis of GTP bound to eIF-2 facilitate 60S association

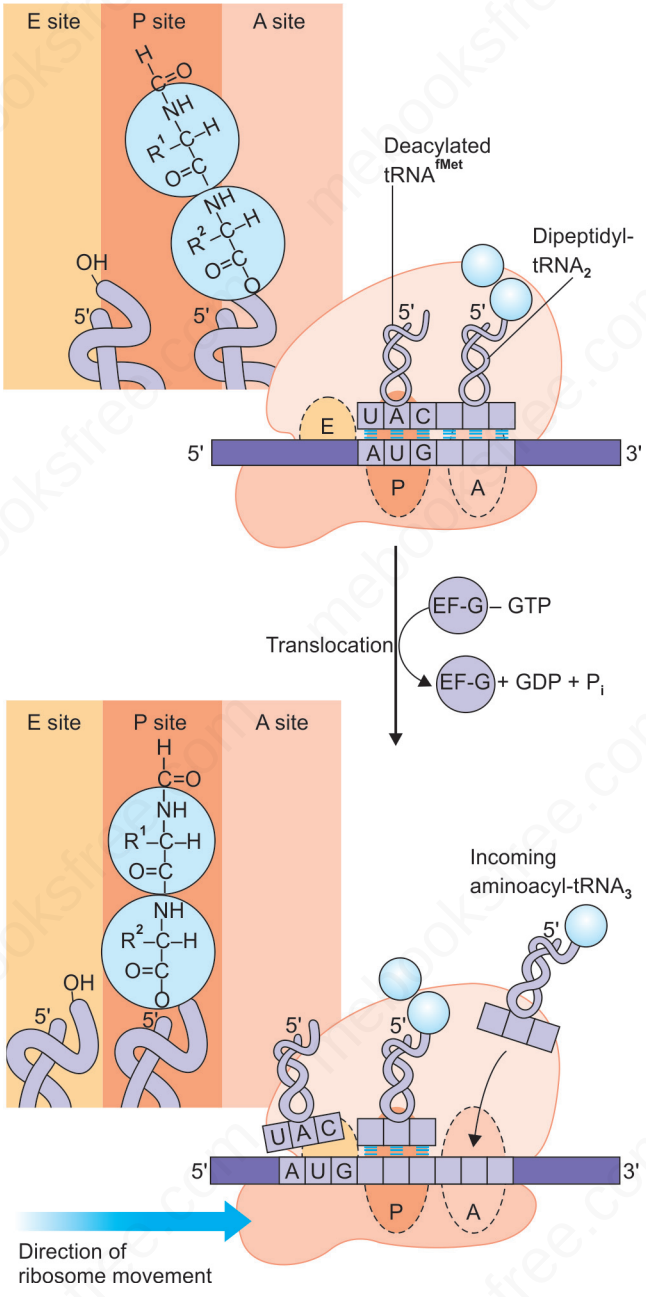


Fig. 12.3: Elongation of translation

Energetics of Peptide Bond Synthesis

Charging of tRNA to $tRNA^{aa}$ – 2 Inorganic Phosphates = 2ATPs

EF1-binding of $tRNA^{aa}$ to A Site- 1GTP

EF2-Translocation-1GTP

No Energy for actual peptide bond synthesis.

Even though the actual peptide bond formation does not require energy, for the formation of one peptide bond hydrolysis of 4 inorganic phosphates is required. (2 ATP for charging of the amino acid 1GTP for EF-1 and 1 GTP for EF-2 Translocation).

Regulation of Translation

The two control points of translation are:

1. eIF 4E of 4F complex.
2. eIF 2.

Rate of Protein Synthesis

Prokaryotes-18 Amino acids per second.
Eukaryotes-6 Amino acids per second

Bacterial Protein synthesis Inhibitors

Reversible: Bacteriostatic

- Tetracyclins
- Chloramphenicol
- Erythromycin and Clindamycin.

Irreversible Inhibitors-Bactericidal

- Streptomycin and other aminoglycosides.

Mammalian Protein Synthesis Inhibitors

Inhibitor	Mechanism of Action
Puromycin[Q]	Structural analog of tyrosinyl tRNA
Cycloheximide	Inhibit peptidyltransferase in the 60S ribosomal subunit
Diphtheria toxin[Q]	Exotoxin of Corynebacterium diphtheriae Catalyzes ADP ribosylation of: Elongation Factor-2 on the unique amino acid diphthamide in mammalian cell This inactivates EF-2, specifically inhibits mammalian protein synthesis
Ricin[Q]	From castor bean inactivates eukaryotic 28S ribosomal RNA

Post–translational Modifications

- Covalent modifications of aminoacyl residues
- Gamma carboxylation
- Hydroxylation
- Methylation
- Glycosylation
- Zymogen activation.

Polysome or Polyribosome

- Multiple ribosome on the same mRNA are called Polysome or Polyribosome

REVIEW QUESTIONS

1. A codon consists of: (AIIMS 90, UP 99, WB 02)
a. One molecule of aminoacyl-tRNA
b. Two complementary base pairs
c. 3 consecutive nucleotide units
d. 4 individual nucleotides

Ans. c. 3 consecutive nucleotides.
(Ref: Harper 30/e page 415)

- Each codon consists of a sequence of three nucleotides, i.e. it is a triplet code.

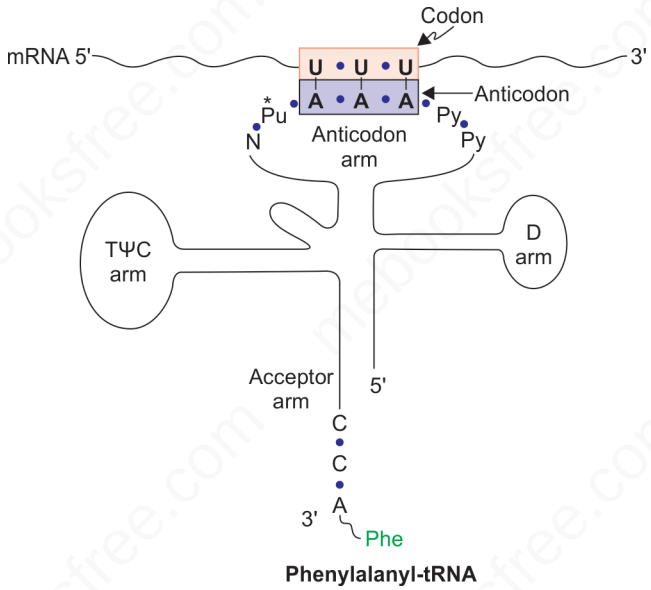
2. All are true of genetic code except:
a. Degenerate (DNB 2001, Delhi 98, TN 95)
b. Universal
c. Punctuation
d. Nonoverlapping

Ans. c. Punctuation. *(Ref: Harper 30/e page 415)*

Features of the Genetic Code
- Degenerate
- Unambiguous
- Nonoverlapping
- Not punctuated
- Universal.

3. Wobble hypothesis – regarding the variation true is: (PGI Dec 07)
a. 3-end of anticodon
b. 5-end of anticodon
c. mRNA
d. tRNA

Ans. b. 5-end of anticodon. *(Harper 30/e page 416)*



5′ end of anticodon suggesting the binding of 3rd nucleotide of codon and anticodon is not strictly obeying the Watson–Crick base pairing rule. This is called Wobble. It is between nucleotide in the 3′ end of codon and 5′ end of anticodon.

4. Components of 50s subunit is/are: (PGI May 2011)
a. 23S
b. 28S
c. 5S
d. 5.8S
e. 16S

Ans. a, c. 23S, 5S. *(Ref: Harper 30/e page 367)*

Ribosomal Assembly in Prokaryotes
70S Ribosome = 30S + 50S Subunits
In 30S Subunit 16S rRNA + Proteins
In 50S rRNA 23S rRNA + 5S rRNA + Proteins.

Ribosomal Assembly in Eukaryotes
80S Ribosome = 40S Subunit + 60S Subunit
60S Subunit = 28S rRNA + 5.8S rRNA + 5S rRNA + ~50 proteins
40S = 18S rRNA + ~30 proteins.

5. Ribosome 60S Subunit contains: (PGI Nov 2009)
a. 5.8S subunit
b. 23S subunit
c. 28S subunit
d. 16S subunit
e. 18S subunit

Ans. a, c. 5.8S Subunit, 28S Subunit.
(Ref: Harper 30/e page 367)

6. There are 20 amino acids with three codons in spite of the number of amino acids could be formed is 64 leading to that an amino acid is represented by more than one codon is called: (AIIMS May 2012)
a. Transcription
b. Degeneracy
c. Mutation
d. Frame shift

Ans. b. Degeneracy. *(Ref: Harper 30/e page 415)*

Genetic Code
The whole set of codons representing all the amino acids is called Genetic Code.

Cracking of Genetic Code was done by Marshal Nirenberg and Har Gobind Khorana

Two amino acids with single codon

AUG ---- Methionine

UGG ---- Tryptophan.

Salient Features of Genetic Code

1. **Triplet Codon**: Each amino acid is represented by triplet sequence.
2. Degenerate (Redundant)
   More than 1 codon represent a single aminoacid.
   Degeneracy of the codon lies in the **3rd Base**.
3. Nonoverlapping
   Reading of genetic code does not involve overlapping sequence.
   Amino Acids with maximum number of codons (Six codons).
   Serine, Arginine, Leucine
4. Unambiguous
   A codon can represent only one amino acid.
5. Universal
   A specific codon represent a specific amino acid in all the species.
   Exception to this rule–Codons of Mitochondrial DNA
6. Initiator codon
   In Eukaryotes --- AUG codes for Met
   In Prokaryotes --- AUG codes for N-Formyl Methionine.
7. Terminator Codons
   UAG --- Amber
   UGA --- Opal
   UAA----Ochre.

Exceptions:

- UGA can be recoded to Selenocysteine
- UAG can be recoded to Pyrrolysine
- UGA codes for Tryptophan in mitochondrial DNA.

8. Wobbling Phenomenon
   The base pairing at the 3rd nucleotide between the anticodon in the tRNA and Codon in the mRNA is not stringently regulated.

Q. 7. Genetic code has triplet of nucleotides each for one amino acid. When an amino acid is specified by more than one codon, it is called: **(AIIMS Nov 2012)**

a. Transcription
b. Degeneracy
c. Mutation
d. Frameshift

Ans. b. Degeneracy. *(Ref: Harper 30/e page 415)*

8. The polypeptide from poly (A) is: **(AIIMS Nov 2012)**

a. Polylysine
b. Polyglycine
c. Polyproline
d. Polyalanine

Ans. a. Polylysine. *(Ref: Harper 30/e page 415)*

Poly A codes for Lysine

Poly C codes for Proline

Poly G codes for Arginine

Poly U codes for Phenylalanine.

9. If constitutive sequence of 4 nucleotide codes for 1 amino acid, how many amino acids can be theoretically formed? **(Nov 2012)**

a. 4
b. 64
c. 16
d. 256

Ans. d. 256. *(Ref: Harper 30/e page 415)*

Codon

The triplet nucleotide sequence present in the mRNA representing specific amino acid

If 1 base represent 1 amino acid only 4 amino acid.

If 2 base represent 1 amino acid 4^2 Amino acids, i.e. 16 amino acids.

If 3 base 4^3, i.e. 64 amino acids.

If 4 bases 4^4, i.e. 256 amino acids.

10. False about eukaryotic protein synthesis: **(AIIMS May 2009)**

a. N formyl Met is the first tRNA to come into action
b. mRNA read from 3′ to 5′
c. eIF-2 shifts between GDP to GTP
d. Capping helps in attachment of mRNA to 40S ribosome

Ans. Both a and b. *(Ref: Harper 30/e page 422)*

N formyl Met is the first tRNA to come into action

mRNA read from 3′ to 5′.

Explanation

Option a

First tRNA to come into action is methionyl tRNA in eukaryotes and N Formyl tRNA in prokaryotes.

Option b

The translation of the mRNA commences near its 5′ terminal with the formation of the corresponding amino terminal of the protein molecule.

The message is read from 5′–3′ for translaton.

Option d

4E subunit of eIF–4F that is bound to the cap forms a circular structure that helps direct the 40S ribosomal subunit to the 5′ end of the mRNA.

The poly (A) tail stimulates recruitment of the 40S ribosomal subunit to the mRNA.

Both Poly A tail and 5′ cap is needed for the attachment of mRNA to the 40S Subunit.

11. Termination process of protein synthesis is performed by all except: (PGI May 2010)

a. Releasing factor
b. Stop codon
c. Peptidyltransferase
d. UAA codon
e. AUG codon

Ans. e. AUG codon. *(Ref: Harper 30/e page 423)*

Termination of Protein Synthesis

Stop codons specifies the sites to stop translation. *Releasing factor-1* along with Releasing factor 3 helps in the termination

Hydrolysis of GTP to GDP

Peptidyltransferase also helps in the hydrolysis of the polypeptide chain.

12. True about Ribozyme (AIIMS Nov 2012)

a. Peptidyltransferase activity
b. Cuts DNA at specific site
c. Participate in DNA synthesis
d. GTPase activity

Ans. a. Peptidyltransferase activity. *(Ref: Harper 30/e page 411)*

Ribozyme is RNA with catalytic activity.

- E.g.: Sn RNA in Spliceosome–Takes part in splicing of exons and removal of introns
- Ribonuclease P- Cuts the RNA
- **Peptidyltransferase**: Peptide Bond formation.

Other options

Cuts the DNA at specific site—Restriction Endonuclease

DNA Synthesis—DNA Polymerase.

13. Part of eukaryotic DNA contributing to polypeptide synthesis (PGI May 2011)

a. Exon
b. Enhancer
c. Leader sequence
d. tRNA
e. ncRNA

Ans. a. Exon.

14. Stop codons are: (PGI Nov 2010)

a. UAA
b. UAG
c. UGA
d. UAC
e. UCA

Ans. a, b, c. UAA, UAG, UGA. *(Ref: Harper 30/e page 415)*

Terminator Codons/Stop Codons/Nonsense Codons

UAG --- Amber

UGA --- Opal

UAA --- Ochre.

Exceptions

UGA can be recoded to Selenocysteine.

UAG can be recoded to Pyrrolysine.

UGA codes for Tryptophan in mitochondrial DNA.

15. Met-tRNA would recognize: (PGI Nov 2009)

a. AUG
b. GCA
c. GUA
d. UAC
e. GAC

Ans. a. AUG. *(Ref: Harper 30/e page 415)*

The first AUG sequence after the marker sequence is defined as the start codon. AUG codon binds with Met $tRNA^1$.

16. Which of the following statement is true? (Ker 2008)

a. N-formylmethionine is the precursor of eukaryotic polypeptide synthesis
b. Eukaryotic ribosomes are smaller than prokaryotic
c. Identification of 5′ cap of mRNA by IF4E is the rate limiting step
d. Elongation factor 2 shuttles between ADP and ATP

Ans. c. Identification of 5′ cap of mRNA by IF4E is the rate limiting step. *(Ref: Harper 30/e page 422)*

Rate limiting steps of Protein Translation are:
eIF 2
eIF 4F (specifically saying 4E of 4 F complex)

Option a: Methionine is the first amino acid in eukaryotic protein synthesis

Option b: Eukaryotic Ribosome is bigger than Prokaryotic ribosome

Option d: Elongation Factor 2 is associated with hydrolysis of GTP not ATP.

17. RNA polymerase differs from DNA polymerase (Ker 2007)

a. It edits and synthesis
b. Synthesize RNA primers
c. Synthesis only in 5 to 3 direction
d. Uses RNA templates

Ans. b. Synthesize RNA primer.

(Ref: Harper 30/e page 415)

- Option a: RNA Polymerase do not have editing function
- Option c: Both DNA Polymerase and RNA Polymerase synthesize in 5′ to 3′ direction
- Option d: Both use DNA as template
- Option b: RNA primer is synthesized by RNA Polymerase.

Comparison between DNA Polymerase and RNA Polymerase	
DNA Polymerase	RNA Polymerase
Involved in DNA Synthesis (Replication)	Involved in RNA Synthesis (Transcription)
Needs a primer	Do not need a primer
Synthesize in 5' to 3' direction	Synthesize in 5' to 3' direction
Has DNA repair and proof-reading activity	Do not have DNA repair and proofreading activity

18. The cellular component for protein synthesis is: (AI 97)

a. Smooth endoplasmic reticulum
b. Rough endoplasmic reticulum
c. Ribosomes
d. Mitochondria

Ans. c > b. Ribosome > RER.

Rough endoplasmic reticulum is ER studded with Ribosome, there also in Ribosome protein synthesis takes place.

19. Amber codon refers to: (AIIMS May 01)

a. Mutant codon
b. Stop codon
c. Initiating codon
d. Codon for more than one amino acids.

Ans. b. Stop codon *(Ref: Harper 30/e page 415)*

- Amber is UAG stop codon
- Ochre is UAA stop codon
- Opal is UGA stop codon.

20. Shine-Dalgarno sequence in bacterial mRNA is near: (AI 2004)

a. AUG codon
b. UAA codon
c. UAG codon
d. UGA codon

Ans. a. AUG codon. *(Ref: Harper 30/e page 415)*

Shine-Dalgarno sequence is the marker sequence. The first AUG codon after Shine-Dalgarno sequence is the start codon in bacteria. Similarly in eukaryotes there is Kozak sequence.

21. True regarding aminoacyl tRNA synthetase is A/E: (SGPGI 06)

a. Is accepting tRNA
b. Implement genetic code
c. Attachment of amino group to 5′ end of tRNA
d. Editing function

Ans. c. Attachment of amino group to 5′ end of tRNA.

(Devlin 7/e page 216)

The reaction of Aminoacyl tRNA synthetase
Amino acid + ATP + Enzyme → Amino acid-AMP-Enzyme complex + PPi
Amino acid - AMP - Enzyme complex + tRNA → Aminoacyl tRNA + AMP + Enzyme.

- Aminoacyl tRNA synthetase first accept ATP and Amino acid
- Then the enzyme accepts the specific tRNA
- Amino acid is attached to 3′ hydroxyl adenosyl end of tRNA
- Two inorganic phosphates are used
- This enzyme is a part of editing mechanism of translation.

22. In translation process, proofreading of mRNA is done by: (AIIMS Dec 97)

a. RNA polymerase
b. Aminoacyl tRNA synthetase
c. Leucine zipper
d. DNA

Ans. b. Aminoacyl tRNA synthetase.

(Ref: Harper 30/e page 415)

- Amino acyl tRNA synthetase which recognises the specific tRNA is a proofreading mechanism.

23. Which enzyme involved in translation is often referred to as 'Fidelity enzyme': (AI 1998)

a. DNA polymerase
b. RNA polymerase
c. Aminoacyl tRNA synthetase
d. Aminoacyl reductase

Ans. c. Aminoacyl tRNA synthetase.

(Ref: Harper 30/e page 415)

24. The hydrolytic step leading to release of polypeptide chain from ribosomes is catalyzed by: (PGI JUNE 02)

a. Stop codons
b. Peptidyl transferase
c. Releasing factors
d. AUG codon
e. Dissociation of ribosomes

Ans. b, c. Peptidyl transferase, Releasing factors.

(Ref: Harper 30/e page 423)

- Releasing factor RF1 recognises that a stop codon resides in the A site
- RF1 bound by a complex consisting of releasing factor RF3 bound by GTP
- This complex, along with peptidyltransferase catalyses the hydrolysis of bond between peptide and tRNA occupying the P site
- Stop codon is not catalysing the hydrolytic cleavage, so it cannot be the answer.

25. About peptidyltransferase true is: (JIPMER 2000)

a. Used in elongation and cause attachment of peptide chain to A- site of tRNA
b. Used in elongation and cause attachment peptide chain to P site
c. Used in initiation and cause 43S complex formation
d. Used in initiation and cause 48S complex formation

Ans. a. Used in elongation and cause attachment of peptide chain to A-site of tRNA.

(Ref: Harper 30/e page 422)

- The α amino group of the new aminoacyl-tRNA in the A site carries out a nucleophilic attack on the esterified carboxyl group of the **peptidyl-tRNA** occupying the **P site (peptidyl or polypeptide site)**. At initiation, this site is occupied by the initiator met-tRNAi
- This reaction is catalyzed by a **peptidyltransferase**, a component of the 28S RNA of the 60S ribosomal subunit
- This is another example of ribozyme activity.

26. Termination is caused by all except: (AIIMS Dec 90)

a. RF-1
b. UAA
c. Peptidyltransferase
d. 48S complex

Ans. d. 48S complex. *(Ref: Harper 30/e page 419)*

Termination is helped by RF-1, RF-3, stop codon and peptidyltransferase.

27. True about translation of protein is: (PGI Dec 98)

a. It has 3 steps: initiation, elongation, termination
b. IF-2 prevent reassociation of ribosomal subunit
c. IF-3 and 1A cause binding of initiation factors
d. IF 2 has α and β units

Ans. a, c. It has 3 steps …, IF-3 and 1A cause binding …

(Ref: Harper 30/e page 419)

- Like transcription, protein synthesis can be described in three phases, Initiation, Elongation and Termination
- eIF3 and eIF 1A bind to newly dissociated 40S ribosomal unit and prevent its reassociation with 60S subunit till translation initiation factors are all associated
- eIF-2 is a part of binary complex
- eIF-2 consist of α, β and γ subunits
- eIF is one of the control points of translation.

28. 43S preinitiation complex include all except: (AIIMS Dec 93)

a. IF-3
b. IF-1A
c. IF-2
d. IF-4F

Ans. c. IF-2 *(Ref: Harper 30/e page 419)*

43 S Preinitiation complex consist of

- GTP-eIF2-tRNAi + 40S subunit
- This 43 S Preinitiation complex is stabilized by eIF-3 and eIF-1A.

29. IF 4F include all except: (PGI June 97, JIPMER 99)

a. 4A
b. 4G
c. 4E
d. 4S

Ans. a, b, c. 4A, 4G, 4E.

(Ref: Harper 30/e page 422)

- Capbinding complex consists of 4A, 4G, 4E.

30. For 1 peptide bond formation how many high energy phosphate bonds are required? (PGI Dec 07)

a. 0
b. 1
c. 2
d. 3
e. 4

Ans. a. 0. *(Ref: Harper 30/e page 422)*

For peptide bond formation no energy is required as the aminoacyl tRNA is already activated.

31. Vitamin required for post-translational modification of coagulants is: (AI 97)

a. Vitamin A
b. Vitamin C
c. Vitamin B6
d. Vitamin K

Ans. d. Vitamin K.

Gamma carboxylation of Clotting factors require Vitamin K.

13 Regulation of Gene Expression

Topics Included

- Regulation of Gene Expression at Different Levels
- Lac Operon
- Epigenetics
- miRNA and SiRNA
- Mitochondrial DNA
- Mutation
- Patterns of Inheritance
- DNA Polymorphisms

Gene Expression

- Organisms adapt to the environmental changes by altering gene expression
- Most common regulation is modulation of transcription.

Housekeeping Gene (Constitutive Gene)

- Genes which are expressed at a constant rate in almost all the cells of the body
- Required for the basal cellular function, e.g. Enzymes for glycolysis.

Inducible Gene

- Genes which are expressed under special circumstances.
- Increase in response to an activator or inducer.
- Decrease in response to a repressor.

Regulation of Gene Expression at Different Levels

At the level of Transcription

- Induction and Repression (Explained by Operon Concept)
- Alternate mRNA Processing (Described in Chapter Transcription)
- RNA Editing (Described in Chapter Transcription)
- mRNA Stability
- Noncoding RNA-induced regulation (Described in Chapter Transcription)
- Epigenetic modifications.

At the level of DNA

- Gene Amplification
- Gene Rearrangement
- Transposition of DNA.

Operon Concept

- Lactose Operon by Francois Jacob and Jacques Monod in 1961
- Operon is the linear array of genes involved in a metabolic pathway, e.g. Lac operon concerned with lactose metabolism in prokaryotes.

Lac Operon Comprises of

i. **Three structural genes coding for 3 proteins:**
   - Lac z—Beta galactosidase
   - Lac y—Permease-a carrier protein that helps permeation of lactose to cell
   - Lac a—Thiogalactoside Transacetylase – function not known.

ii. **Regulatory genes comprises of:**
   - Lac promoter
   - Lac operator
   - Lac I encodes lac operon repressor protein.

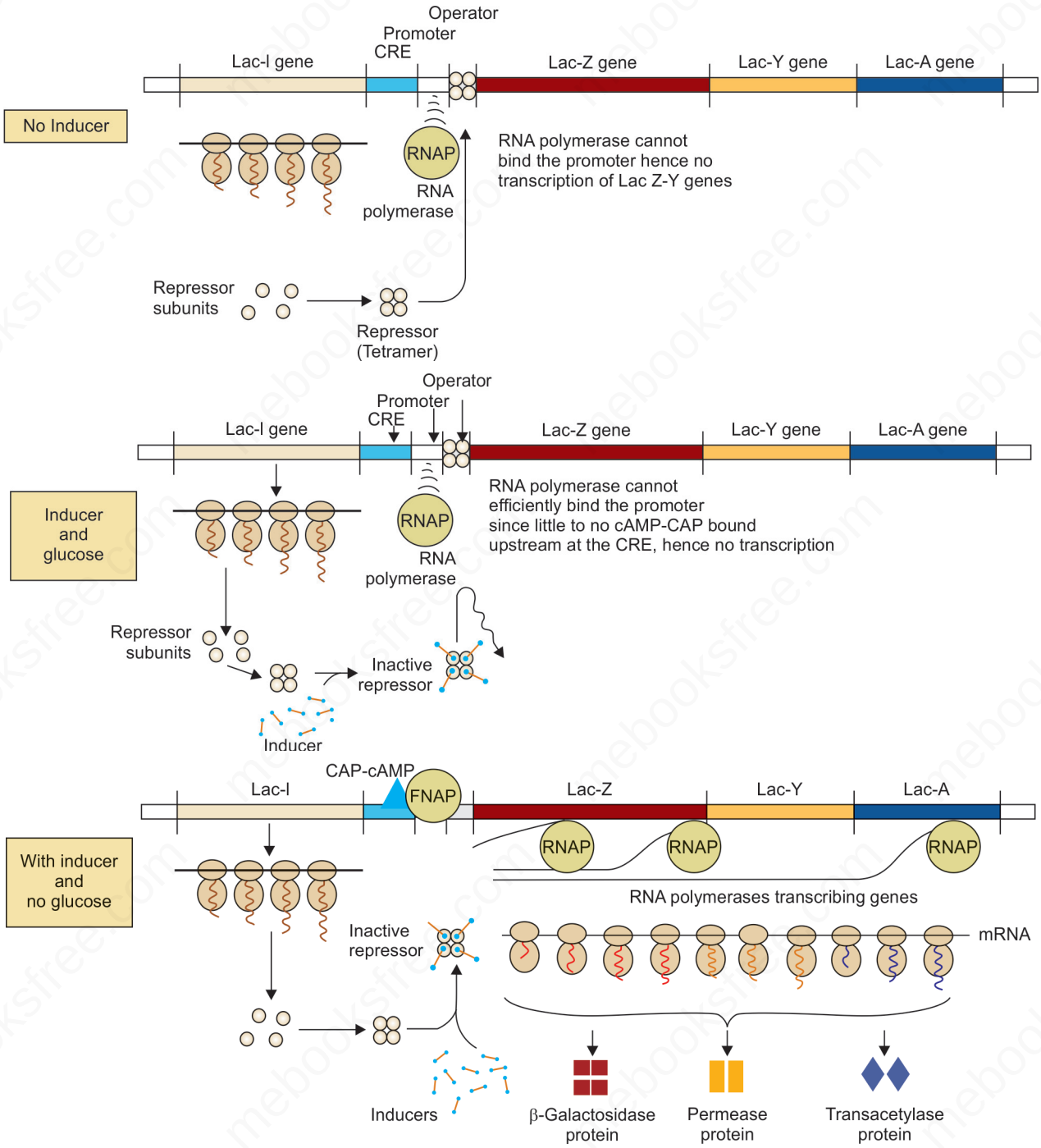


Fig. 13.1: Lac operon

Role of Catabolite Activator Protein (CAP) in Lac Operon

CAP is a Positive Regulator of Lac Operon

- If glucose concentration low-cAMP level is high
- CAP is complexed with cAMP
- CAP-cAMP Complex facilitates binding of RNA Polymerase to promoter
- Structural genes expressed.

When Glucose Present and Lactose Absent

- Lac I ----> Repressor Protein active
- Binds to the Operator Site
- RNA Polymerase cannot move to Promoter site
- CAP is inactive as cAMP level is low
- Lac Operon Repressed, Structural genes are not transcribed.

When Glucose Absent and Lactose Present

- Lac I ---> Repressor protein
- The repressor molecule, which has got affinity to lactose
- This brings a conformational change in the repressor molecule and it can no more bind to the operator site
- CAP is active as cAMP level is high
- Hence, structural genes are transcribed (De-repression).

When Both Glucose and Lactose Absent

- CAP inactive as cAMP
- Hence, structural gene not transcribed
- Repression of Lac operon.

Gratuitous Inducer

- Lactose analogs capable of inducing Lac operon, e.g. Isopropyl Thiogalactoside (IPTG).

Concept

- Whenever Glucose is present irrespective of the presence or absence of lactose, the lac operon is switched off.
- Whenever Glucose is absent lac operon is on.

GENE AMPLIFICATION

- Gene amplification is the process by which number of gene available for transcription is increased (Fig. 13.2), e.g. Person who is on Methotrexate develop resistance to MTX by increasing the number of genes for Dihydrofolate Reductase.

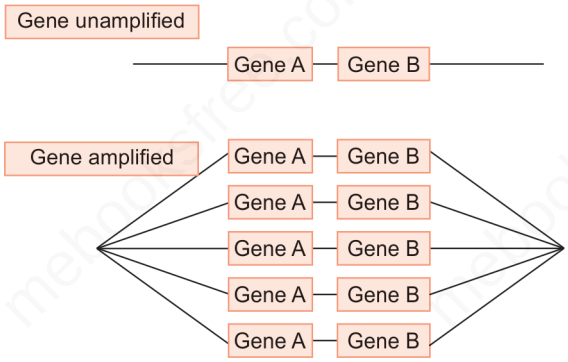


Fig. 13.2: Gene amplification

GENE REARRANGEMENT

- Different gene segments are brought together in different combination is called Gene rearrangement (Fig. 13.3).
- The IgG light chain is composed of variable (V_L), joining (J_L), and constant (C_L) domains or segments.
- For particular subsets of IgG light chains, there are roughly 300 tandemly repeated V_L gene coding segments, 5 tandemly arranged J_L coding sequences, and roughly 10 C_L gene coding segments.

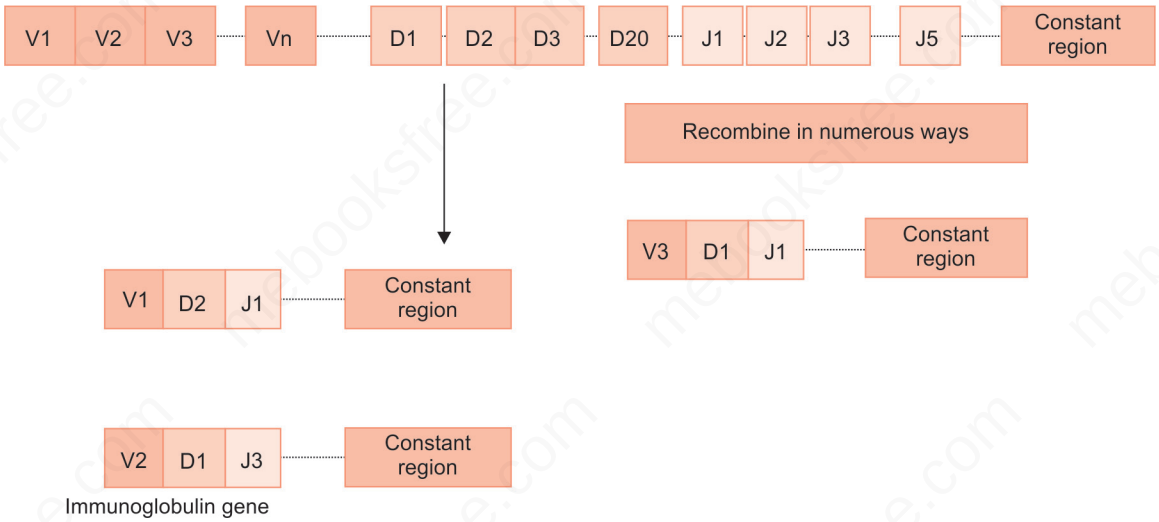


Fig. 13.3: Gene rearrangement in immunoglobulin

- During B-Lymphocyte development, different VDJ segments combine to form *unique variable region in immunoglobulin*.
- This allows generation of 10^9-10^{11} different immunoglobulin from single gene.

TRANSPOSONS (JUMPING GENE)

- DNA sequences that move to new positions within the genome
- Discovered by Barbara McClintock.
- Transposase enzyme help in this process.

Retroposons

- DNA sequence move from one segment to another through an RNA Intermediate
- DNA segment is converted to RNA, RNA moves to another location, where it is reversely transcribed to a DNA.

GENE SWITCHING

The process by which one gene is switched off while a closely related gene take up its function, e.g. during primary immune response genes for IgM is active but during secondary immune response genes for IgG is active.

GENE SILENCING

- The process by which a gene is switched off
- By various epigenetic mechanisms (described later)
- By RNA interference (RNAi) by nc RNAs (described in chapter Transcription).

REGULATION OF mRNA STABILITY

- mRNA exist in cytoplasm as Ribonucleoprotein particle (RNP)
- Much of the mRNA metabolism likely to occur in P bodies.

RNP can help mRNA in two ways:

- Proteins protect mRNA from Nuclease
- It can promote nuclease attack

mRNA stability is determined by

1. 5′ cap that prevents 5′ exonuclease attack
2. 3′ Poly A tail prevent 3′ exonuclease attack
3. Other structures in coding region, 5′ Untranslated region, 3′ Untranslated regions of mRNA
4. Stem loop structure in 3′ end region prevents exonuclease attack in histone mRNA which lack Poly A tail
5. Stem loop structure in 3′ end is critical for regulation of mRNA encoding transferring receptor
6. Presence AU rich region in the 3′ UTR of certain mRNA shortens its half life of certain mRNA
7. Seed sequence in the 3′ UTR determine the specificity of binding of miRNA to mRNA.

EPIGENETICS[Q]

Reversible heritable chemical modification[Q] of DNA or histone or nonhistone proteins that does not alter DNA sequence itself.

- The term epigenetics means, above genetics' as the nucleotide sequence is unaltered
- This is one of the recently discovered method of regulation of gene expression.

Epigenome: Constellation of covalent modification of DNA and histones that impact chromatin structure and modulation of gene expression.

Epigenetic modifications include

- DNA Methylation
- Post-translational modification of Histones.

Functional Consequences of Chemical Modification of DNA

DNA Methylation and demethylations

- DNA methylation is usually restricted to Cytosine residues in CpG dinucleotide of CpG islands
- Enzyme responsible for methylation is Methyl transferases
- DNA methylation generally decreases the gene expression or gene silencing
- Acute DNA demethylation, increases the rate of transcription.

Gene promoters and regulation by methylation-demethylations

- Majority of gene promoters have high CG content.
- CpG islands are the common sites of methylation-demethylations
- Hence, methylation-demethylations plays an important role in regulation of gene expression.
- CpG islands of promoters are typically unmethylated, it favors transcription.

Histone Covalent Modifications

- Also known as "The Histone code."
- Wide range of Post translational modifications.
- They are dynamic and reversible.

Histone Acetylation and Deacetylation

- Histone acetylation and deacetylation is the best understood modification of histones
- Acetylation occurs on lysine residues in the amino terminal tails of histone molecules
- Histone acetylation increases the gene transcription
- Histone Acetyltransferase (HAT) acetylate the histones
- Histone deacetylases (HDAC) deacetylate the histones.

Mechanism of histone acetylation and deacetylation

- Histone acetylation reduces the positive charge of amino terminal tails of histone molecules.
- Decreases the binding affinity for the negatively charged DNA.
- This increases euchromatin formation.
- Transcription factors can bind to the promoters.
- Histone deacetylation has the opposite effect, i.e. decreases transcription and increases heterochromatin formation.

Histone methylation

- Addition of methyl group to specific lysine residues, but rarely to arginine residues
- Histone methyltransferases is the enzyme.

Functional consequence—Alter chromatin configuration, which favor or decrease transcription.

Histone phosphorylation

- Serine residues can be modified by phosphorylation;
- Functional consequences—Depending on the specific residue the DNA may be opened up for transcription or condensed to become inactive.

Other Histone Modification

Histone modification	Possible role of the modification
Acetylation of core histones[Q]	Increases transcription and increased expression of the gene
Phosphorylation of histone H1	Condensation of chromosomes during the replication cycle.
ADP-ribosylation of histones	DNA repair
Methylation of histones	Activation and repression of gene transcription.
Monoubiquitylation	Gene activation, repression, and heterochromatic gene silencing.
Sumoylation of histones (SUMO; small ubiquitin-related modifier)	Transcription repression

Biochemical Functions of Epigenetic Modification[Q]

1. Regulation of tissue specific gene expression
2. X chromosome inactivation (Facultative Heterochromatin) one of the two X-chromosomes in every cell of a female
3. Genomic imprinting: Gene inactivation on selected chromosomal regions of autosomes is called Genomic Imprinting. This is the cause of preferential expression of one of the parental allele.

   Maternal imprinting refers to transcriptional silencing of the maternal allele.

   Paternal imprinting implies that the paternal allele is inactivated.

   Imprinting occurs in the ovum or the sperm, before fertilization.
4. Ageing Process

Epigenetic Changes Causing Pathological Alteration

1. Fragile X syndrome
   - Promoter site hypermethylation causing FMR-1 gene silencing
2. Cancer
   - *DNA methylation and histone modifications* dictate which genes are expressed, which in turn determines the lineage commitment and differentiation state of both normal and neoplastic cells
   - Local promoter hypermethylation of tumor suppressor gene leads decreased expression of the tumor suppressor gene.
3. DNA methylation is considered as a defence mechanism that minimise the expression of retroviral incorporated sequences
4. Genomic Imprinting

Prader-Willi and Angelman Syndrome

The molecular basis of these two syndromes lies in the Genomic Imprinting.

Prader- Willi Syndrome

- A gene or set of genes on maternal chromosome 15q12 is imprinted (and hence silenced)
- The only functional allele (s) are provided by the paternal chromosome.

Causes of Prader-Willi Syndrome

- Paternal deletion of Prader-Willi locus on located on Chr 15 (Most common cause)

- Maternal uniparental disomy
- Rarely due to imprinting defect in paternal chromosome.

Angelman Syndrome

- A distinct gene that also maps to the same region of chromosome 15 is imprinted on the paternal chromosome.
- Only the maternally derived allele of this gene is normally active.

Causes of Angelman Syndrome

- Maternal deletion of corresponding locus on located on Chr 15 (Most common cause)
- Paternal uniparental disomy
- Rarely due to imprinting defect in maternal chromosome.

Some Examples of Tumor Suppressor Gene Silencing by Epigenetic Mechanism

- CDKN2A, a complex locus that encodes two tumor suppressors, p14/ARF and p16/INK4a p14/ARF is epigenetically silenced
- Colon and Gastric cancers.p16/INK4a is silenced in a wide variety of cancers like
- Bladder cancer, Head and Neck Cancer, ALL, Cholangiocarcinoma
- BRCA-1 Silencing–Cancer Breast
- VHL Silencing-Renal cell Cancer
- MLH-1 Silencing-Colon Cancer.

Molecular Methods to Detect Epigenetic Modifications in the Genome

Traditional Sanger sequencing alone cannot detect epigenetic modification.

1. Methylation-specific PCR can detect DNA methylations.
2. DNA Chromatin Immunoprecipitation (ChIP) followed by Microarray hybridization analysis or Direct Sequencing (ChIP-Chip or ChIP-Seq) detect histone modifications.
3. Bisulphite Sequencing.
4. Methylation Sensitive Restriction Endonuclease Digestion.

Principle of Bisulphite Sequencing

- Sodium bisulphite convert unmethylated cytosine to uracil, which function like thymine in base pairing.
- But methylated cytosines are protected from modification, hence remain unchanged.

Procedure

- Treat genomic DNA with Sodium Bisulphite.
- Thus, unmethylated (modified) DNA is discriminated from the methylated (unmodified) DNA on the basis of sequence analysis.

CHROMATIN IMMUNOPRECIPITATION (ChIP)

This method allows precise localization of a particular protein or modified protein on the DNA, e.g. acetylated, phosphorylated histones, etc.) on a particular DNA sequence element in living cell.

RNA IMMUNOPRECIPITATION (RIP)

- An RNA immunoprecipitation method, performed like ChIP, which is used to score specific binding of a protein to a specific RNA in vivo.
- RIP uses formaldehyde cross linking to induce covalent attachment of proteins to RNA
- CLIP: A method that uses UV cross-linking to induce covalent attachment of distinct proteins to specific RNAs in vivo.

Remember

- ChIP is Chromatin immunoprecipitation
- Chip is hybridization on DNA Chip or Microarray.

Therapeutic Application of Epigenetic Modification

Unlike DNA mutations, epigenetic changes are potentially reversible by drugs that inhibit DNA or histone-modifying factors.

Thus, there is considerable interest in treating cancers with drugs that correct epigenetic abnormalities in cancer cells, with some encouraging early results.

1. Drugs that inhibit DNA Methyltransferases (DNMT inhibitor)
   - Azacytidine
   - 5-aza-2′-deoxycytidine
   - Decitabine
2. Drugs that inhibit histone Deacetylases (HDAC inhibitor)
   - Vorinostat
   - Valproic acid

MITOCHONDRIAL DNA

- 1% of cellular DNA is mitochondrial DNA.
- Mitochondria possess its own DNA and protein synthesizing machinery.
- Human mitochondria contains 2–10 copies of a small circular ~16 kbp dsDNA molecule.
- Composed of Heavy (H) and Light (L) chain or strands.
- Contain 16,569 bp.

Mitochondrial DNA Encodes 37 Structural Genes

They incude

- 2 rRNAs
- 22 mitochondrial tRNAs, large 16S rRNA and small 12S rRNA.
- 13 protein subunits of respiratory chain.Q

- Seven subunits of NADH Dehydrogenase (Complex I)
- Cytochrome b of Complex III
- Three subunits of Cytochrome C Oxidase (Complex IV)
- Two subunits of ATP synthase.

Mitochondria has Unique Genetic Code

Codons[Q]	*Nuclear DNA Code*	*Mitochondrial DNA Code*
AUA	Isoleucine	Methionine
UGA	Stop codon	Tryptophan
AGA, AGG	Arginine	Stop codon

Unique Features of Mitochondrial DNA

1. Mutation rate is very high because
   - No Introns
   - No protective histones
   - No effective repair enzymes
   - It is exposed to oxygen free radicals generated by oxidative phosphorylation.
2. Non-Mendelian type of inheritance
   Cytoplasmic inheritance or Matrilinear inheritance. (Described below)
   Mitochondrial disease with no maternal inheritance
   - Pearson syndrome
   - Kearns-Sayre syndrome (KSS)
3. Heteroplasmy
   - Heteroplasmy is defined as the presence of normal and mutant DNA in different proportions in different cells
   - Mitochondrial disease with homoplasmy
   - Leber's hereditary optic neuropathy
   - Sensorineural deafness

MUTATION

Definition

Any permanent change in the primary nucleotide sequence regardless of its functional consequences.

Mutation rate ~10^{-10}/bp per cell division.

Types of the Mutation

- **Point Mutation**

Single base changes in the nucleotide sequence in the gene.

Base Substitution

Replacement of a single nucleotide by another. These are the most common type of mutation.

i. **Transition**[Q]**:** A purine base replaced by a purine base or a pyrimidine replaced by another pyrimidine.

ii. **Transversion**[Q]**:** A purine base replaced by another pyrimidine, or pyrimidine replaced by another purine.

Effects of Base Substitution

I. Silent mutation
   - If a mutation does not alter the polypeptide product of the gene
   - Also called synonymous mutation

II. Missense mutation
   - The alteration in the nucleotide may result in the incorporation of a different amino acid

   A missense may be:
   i. Acceptable—No clinical symptoms
      e.g. Hb Hikari
   ii. ii. Partially acceptable[Q]
      e.g. Hb S[Q]
   iii. iii. Unacceptable
      e.g. HbM

Another classification of Mis-sense Mutation

1. Conservative mutation
   A missense mutation in which one amino acid is replaced by a similar amino acid
2. Non-conservative mutation
   A missense mutation in which one amino acid is replaced by an amino acid with different characteristics
   e.g. HbS

III. Nonsense mutation
   - A coding codon mutated to a nonsense codon result in premature termination of polypeptide chain.

IV. Splice site mutation
   - Mutation at the splice site results in faulty splicing

V. Promoter site mutation
   - Results in altered gene expression

VI. Frame shift mutation
   - Due to insertion or deletion of nucleotides that are **not a multiple of three** results in frameshift mutation
   - Reading frame is garbled

VII. Null mutation
   - A mutation that leads to no functional gene product is called null mutation

VIII. Constitutive mutation
   - Mutation in which a inducible gene mutated to housekeeping gene or constitutive gene

IX. tRNA suppressor mutation
   - The effect of mutation on mRNA can be suppressed by a mutant tRNA which has a mutant anticodon sequence

– These mutant tRNA which can suppress the effect of mutation are called Supressor tRNAs.

Classes of Mutation in Cystic Fibrosis

Various mutations can be grouped into six "classes" based on their effect on the CFTR protein:

Class I: *Defective protein synthesis*. These mutations are associated with **complete lack of CFTR protein** at the apical surface of epithelial cells.

Class II: Abnormal protein folding, processing, and trafficking. These mutations result in defective processing of the protein from the endoplasmic reticulum to the Golgi apparatus; the protein does not become fully folded and glycosylated and is instead degraded before it reaches the cell surface. **The most common class II mutation is a deletion of three nucleotides coding for phenylalanine at amino acid position 508 (ΔF508)**. Worldwide, this mutation can be found in approximately **70% of cystic fibrosis patients**. Class II mutations are also associated with complete lack of CFTR protein at the apical surface of epithelial cells.

Class III: *Defective regulation*. Mutations in this class prevent activation of CFTR by preventing ATP binding and hydrolysis, an essential prerequisite for ion transport. Thus, there is a normal amount of CFTR on the apical surface, but it is nonfunctional.

Class IV: *Decreased conductance*. These mutations typically occur in the **transmembrane domain of** CFTR, which forms the ionic pore for chloride transport. There is a normal amount of CFTR at the apical membrane, but with reduced function. This class is usually associated with a milder phenotype.

Class V: *Reduced abundance*. These mutations typically affect intronic splice sites or the CFTR promoter, such that there is a reduced amount of normal protein.

Class VI: *Altered regulation of separate ion channels*. Mutations in this class affect the regulatory role of CFTR. In some cases, a given mutation affects the conductance by CFTR as well as regulation of other ion channels. For example, the ΔF508 mutation is both a class II and class VI mutation.

New Mutation and Gonadal Mosaicism

In some autosomal dominant disorders, phenotypically normal parents have more than one affected children.

This is a violation of Mendelian Inheritance.

This is an example of single gene disorder-Non Classic Inheritance.

The sudden unexpected occurrence of a condition due to mutation of a gene is called new mutation.

New mutation occurs post zygotically during early (embryonic) development.

If the mutation affects only cells destined to form the gonads, the gametes carry the mutation, but the somatic cells of the individual are completely normal. Such an individual is said to exhibit *germ line or gonadal mosaicism*.

Thus, a phenotypically normal parent who has germ line mosaicism can transmit the disease-causing mutation to the offspring through the mutant gamete, e.g. Achondrodysplasia, Marfan's syndrome, Neurofibromatosis.

Some Terms used in Genetics

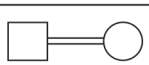
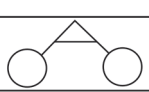
- **Genome:** The complete complement of genetic information in a living organism.
- **Chromosome:** Physical Division of Genome.
- **Genes:** Functional Division of Genome.

Contd...

Contd...

- **Genetic locus:** Location of a particular gene on the Chromosome.
- Alleles
  - In diploid organism there are two sets of chromosomes.
  - Therefore, there are 2 copies of each gene
  - The different forms of the same gene that are found at the same locus are called alleles
  - One allele is received from the father and the other allele from the mother.
  - They are responsible for alternate or contrast character.
- **Genotype** represents the set pattern of gene present in the cell
- **Phenotype** is the observed character expressed by the gene
- **Homozygous:** Both allele are defective.
- **Heterozygous:** One allele is normal and the other allele is defective.
- **Recessive mode of transmission:** Phenotypic expression of the disease only in the homozygous state.
- **Carrier state:** In recessive mode of inheritance if the person carries one abnormal gene it is not phenotypically expressed. Biochemically it is called Trait.
- **Dominant mode of transmission:** Phenotypic expression even when one allele is abnormal or heterozygous state.
- **Autosomal** means defective gene is located in the autosomes. (Somatic)
- **Sex linked** means defective gene is located in the Sex chromosome.

Some Common Pedigree Symbols

Male	
Female	
Unknown sex	
Affected male	
Affected female	
Spontaneous abortion	
Proband	
Heterozygous male	
Heterozygous female	
Female carrier of X linked trait	
Consanguineous union	
Monozygotic twins	
Dizygotic twins	

The Methods to Find the Inheritance Pattern from Pedigree Chart

1. **Autosomal Dominant Inheritance**
   Disorder or trait which is manifested in the heterozygous state.

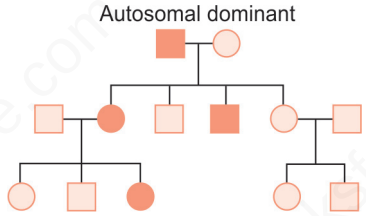


Fig. 13.4: Autosomal dominant inheritance

The characteristics of an autosomal dominant inheritance.

- Males and females are affected in equal proportion.
- Traced through many generation in the family tree. Hence called vertical transmission.
- Usually one of the parents is affected (Exception is new mutation already explained).
- Genetic risk is 50%, i.e. 50% of the progeny will be affected.

Skipping generation

Incomplete penetrance in autosomal dominant inheritance is called skipping generation, i.e. Some individuals inherit the mutant gene but are phenotypically normal.

2. **Autosomal Recessive Inheritance**
   Disorder or trait which manifest in homozygous state.

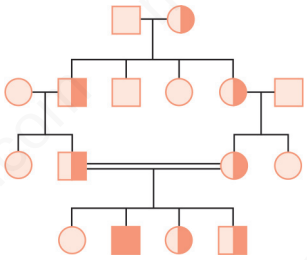


Fig. 13.5: Autosomal recessive inheritance

The characteristics of an autosomal recessive inheritance.

- Males and females are affected in equal proportion
- Affected individuals are usually same generation (Hence called horizontal transmission)
- Consanguineous marriage common
- You can find unaffected parents with affected progeny.

Pseudodominance in autosomal recessive inheritance

- If an individual who is homozygous for an autosomal recessive disorder marry a heterozygous carrier, 50% chance of being affected
- Resemble an autosomal dominant Pedigree.

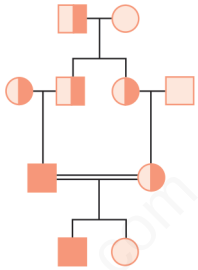


Fig. 13.6: Autosomal recessive with pseudodominance

3. **X-linked Recessive Inheritance**
   Mutant allele present in the X chromosome.

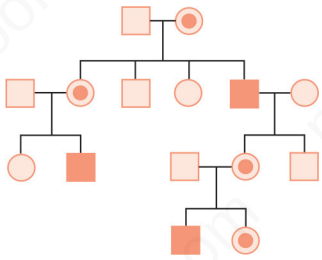


Fig. 13.7: X-linked recessive inheritance

The characteristics of X-linked recessive inheritance.

- Males are usually affected
- Females are usually carriers
- Affected males will have only carrier females
- Carrier females will have affected males
- Male to male transmission is never seen.

Knight move pattern of inheritance

- In X-linked recessive inheritance affected male transmit the mutant allele to carrier female and never to a male
- Carrier female transmit the mutant allele to the male who is affected
- This pattern of transmission is called Knight move pattern or diagonal inheritance.

The difference between hemizygous and homozygous

- In X-linked or Y-linked inheritance, males with mutant allele does not have alternative allele in the homologous chromosome, as there is only one X and one Y chromosome
- Hence, male with mutant allele on X or Y Chromosome is called Hemizygous
- Homozygous means mutant allele is present on both homologous chromosome

4. **X-linked Dominant Inheritance**
   Manifest in heterozygous females and hemizygous males.

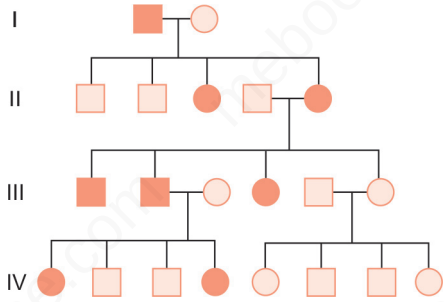


Fig. 13.8: X-linked dominant inheritance

The characteristics of X-linked Dominant Inheritance.

- Resemble autosomal dominant pedigree pattern as 50% chance of being affected
- Affected males transmit the disease to all females of next generation
- Male to male transmission is never seen
- This is because the son will receive only the Y chromosome from the father.

5. Y-Linked Inheritance

- Mutant allele is present in the Y chromosome.
- Other name for Y-linked inheritance is Holandric inheritance.

The characteristics of Y-linked inheritance

- Only males are affected
- Only male to male transmission is seen
- The explanation is simple only Y chromosome carries the mutant allele.

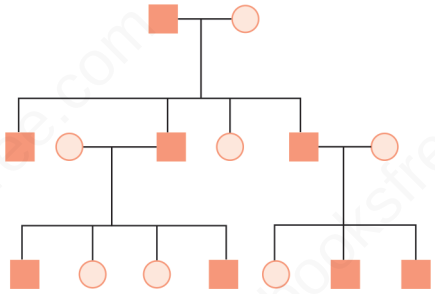


Fig. 13.9: Y-linked inheritance

Y Chromosome carries the genes:

- SRY (Sex Reversal Y) codes, Testis determining factors. Mutated cause, Sex reversal
- DAZ (Deleted in Azoospermia)
- AZF (Azoospermic factor gene) Mutated cause Azoospermia or oligospermia

6. Mitochondrial Inheritance

Mutant allele present in the mitochondrial DNA.

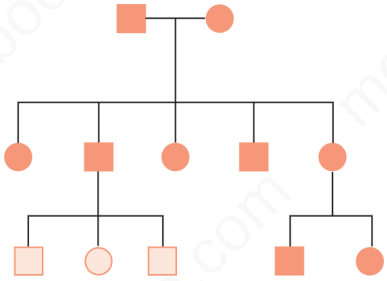


Fig. 13.10: Mitochondrial inheritance

The Characteristics of Mitochondrial inheritance

- Females transmit the disease to all her offsprings
- Males usually never transmit the disease
- This is called Matrilinear inheritance
- Other name is Cytoplasmic inheritance.

Most common inheritance is Autosomal Dominant 65%
Followed by Autosomal Recessive 25% and X-linked Recessive 5%

DNA POLYMORPHISM

- Normal variation in DNA sequence that have a frequency of at least 1% of population
- Any two individuals share 99.9% of DNA sequence.
- DNA variation lies in this 0.1% (1 in 500-1000 bp)
- They are genetic markers that determines the uniqueness of an individual (i.e. like your Fingerprints).

Different Types of Polymorphism

- Single Nucleotide Polymorphism
- Repeat length Polymorphism or Short Tandem Repeats (Variable Number Tandem repeats)
  - Microsatellite repeats
  - Minisatellite repeats
- Copy Number Variations
- Restricted Fragment Length Polymorphism

Single Nucleotide Polymorphism

- DNA variation in single base pair are called SNPs (or Snips)
- Most common polymorphism[Q] (~90% of total polymorphisms)
- No of SNPs in human genome is 10 million
- Occurrence is one nucleotide in every stretch of approximately 100-300 base pairs
- SNPs that are in close proximity are inherited together (are linked) is called haplotype
- Haplotype map information is referred to as Hap map
- May occur anywhere in the genome within the exon, within the intron (most common)

- Used as genetic markers in Linkage and Association Studies.

SNP Genotyping Arrays

- Newer types of genomic arrays are designed to identify single nucleotide polymorphism (SNP) sites genome-wide
- This technology is the mainstay of genome wide association studies.

Repeat length Polymorphism or Short Tandem Repeats or Variable Number Tandem Repeats

- Short repetitive sequence in human DNA is called Repeat length Polymorphism
- Most common one is dinucleotide repeat involve AC to TG on other strand.

Depending on the repeat size it is divided into:

Microsatellite: Repeat size of 2-6 bp.

Total length the repeats extend is usually <1 kbases

Mini satellite: Repeat size of 15-70 bp

Total length the repeats extend is usually 1 to 3 kbases.

Application of Repeat Length Polymorphism

- Useful as genetic markers in Linkage and Association Studies
- Familial diagnosis of disease like Polycystic Kidney Disease
- Cancer genetics
- Paternity testing
- Forensic medicine.

Microsatellite repeat length polymorphism

- The genetic polymorphism ideal for differentiating between two individuals
- The genetic polymorphism ideal to follow the genetic marker transmitted from parent to child
- Extensively used for determining paternity and criminal investigation
- Used for the detection and quantification of transplant chimerism in allogeneic hematopoietic stem cell transplant patients (host and donor).

Method to Detection of Repeat length Polymorphism

- By allele specific PCR

Restricted Fragment Length Polymorphism

- Special type of Polymorphism (Described later in Chapter 13)
- DNA variations that create or abolish a restriction site

Copy Number Variation (CNV)

- Insertion or Deletion of a segment of genome (involve 1 kb to several Mbs)
- About 1500 CNVs detected so far
- Involve substantial regions of the genome, not single nucleotide
- De novo CNVs observed among monozygotic twins
- More recently detected
- 50% occur in the coding regions
- Responsible for human phenotypic diversity.

VUS (Variant of Unknown Significance)

- Sequence alteration which are unclear whether it is a mutation or polymorphism are called Variant of Unknown Significance.

REVIEW QUESTIONS

Operon Concept

1. Which of the following are situated away from the coding region: (PGI June 06)

a. Promoter
b. Enhancer
c. Operator
d. Structural gene

Ans. b. Enhancer *(Ref: Harper 30/e page 401)*

Properties of Enhancers[Q]

- Can be located upstream or downstream of the transcription site
- Work when located long distances from the promoter
- Work when upstream or downstream from the promoter
- Work when oriented in either direction
- Can work with homologous or heterologous promoters
- Work by binding one or more proteins
- Work by facilitating binding of the basal transcription complex to the cis-linked promoter.

2. Housekeeping genes are: (JIPMER 02, WB 03)

a. Inducible
b. Required only when inducer is present
c. Mutant
d. Not regulated

Ans. d. Not Regulated

(Ref: Harper 30/e page 430)

- The expression of some genes is constitutive, meaning that they are expressed at a reasonably constant rate and not known to be subject to regulation. These are often referred to as housekeeping genes
- An **inducible gene** is one whose expression increases in response to an inducer or activator, a specific positive regulatory signal.

3. True among all is (PGI 91)

a. Repressor is dimer and a positive regulator
b. CRP is gratuitous inducer
c. Lactose is positive regulator
d. De-repression is due to presence of glucose
e. Catabolite repression is mediated by CRP

Ans. c, d, e. Lactose is positive regulator, De-repression is due to presence of glucose, Catabolite repression is mediated by CRP *(Harper 30/e page 432, 433)*

- Repressor is a tetramer
- Isopropyl Thiogalactoside (IPTG) is a gratuitous inducer
- Although Lactose is present in the cell, as long as glucose is present *E coli* does not activate lac operon. This is called Catabolite Repression. This is because of catabolite gene activator protein complexed with cAMP. This complex is called cAMP Regulatory Protein (CRP)
- Lactose alone is an inducer of lac operon.

4. False statement is: (PGI 90, WB 03)

a. Repressor binds operator gene
b. Regulator genes produce repressor subunits
c. IPTG is inducer but not substrate
d. Regulator gene is inducible

Ans. d. Regulator gene is inducible

(Harper 30/e page 432, 433)

- Structural genes are inducible, not the regulator genes
- Lac I gene produces Repressor subunits
- IPTG is an inducer of lac-operon, but itself is not a substrate, This is called gratuitous inducer.

5. Lac operon transcription is induced by: (Delhi 03, TN 01)

a. Glucose
b. Glucose with inducer
c. Inducer without glucose
d. Both lactose and glucose

Ans. c. Inducer without glucose

(Ref: Harper 30/e page 433)

Lac operon is repressed when, the cell of *E coli* contains:

- Glucose alone
- Glucose and Inducer (lactose)

Lac operon is induced if

- Lactose alone is present.

6. All of the following statements about Lambda phage are true, except: (AI 2009)

a. In Lysogenic phase it fuses with host chromosome and remains dormant
b. In Lytic phase it fuses with host chromosome and replicates
c. Both Lytic and Lysogenic phase occur together
d. In Lytic phase it causes cell lysis and releases virus particles

Ans. c. Both Lytic and Lysogenic phase…

(Ref: Harper 30/e page 432)

When lambda infects an organism of that species, it injects its 45,000-bp, double-stranded, linear DNA genome into the cell.

Depending upon the nutritional state of the cell, the lambda DNA

1. Will either **integrate** into the host genome (**lysogenic pathway**) and remain dormant until activated
2. It will commence **replicating** until it has made about 100 copies of complete, protein-packaged virus, at which point it causes lysis of its host (**lytic pathway**).

   The newly generated virus particles can then infect other susceptible hosts.

   Poor growth conditions favor lysogeny while good growth conditions promote the lytic pathway of lambda growth.

7. True about transposons (PGI Nov 2013)

a. It has no effects on gene expression
b. Also called jumping genes
c. Mediated by enzyme transposase
d. It is called retrotransposon when it involves an RNA intermediate

Ans. b, c, d. Also called jumping ...., mediated by enzyme ..., It is called Retroposons ...

Transposons (Jumping Gene)

- DNA sequences that move to new positions within the genome
- Discovered by Barbara McClintock
- Transposase enzyme help in this process.

Retroposons

- DNA Sequence move from one segment to another through an RNA Intermediate
- DNA segment is converted to RNA, RNA moves to another location, where it is reversely transcribed to a DNA.

Mitochondrial DNA

8. All are true about mitochondrial DNA except (PGI 2014)

a. Contains 37 gene
b. Transmit from mother to offsprings
c. Transmit in classical mendelian fashion
d. Cause Leber hereditary optic neuropathy

Ans. c. Transmit in classical mendelian fashion

Mitochondrial DNA Encodes Structural Genes

For 2 rRNAs

22 mitochondrial tRNAs

13 protein subunits of respiratory chain.

This includes 37 genes.

9. Mitochondrial DNA is: (AI 2006)

a. Closed circular
b. Nicked circular
c. Linear
d. Open circular

Ans. a. Closed circular *(Ref: Harper 30/e page 362)*

Mitochondrial DNA

- Mitochondria possess its own DNA and protein synthesizing machinery
- Human mitochondria contains 2–10 copies of a small circular dsDNA molecule
- Composed of Heavy (H) and Light (L) chain or strands
- Contain 16,569 bp
- That makes up approximately 1% of total circular DNA

DNA Polymorphism

10. The size of microsatellite repeat sequence is: (PGI Nov 2014)

a. Less than 1 kb
b. 2–6 bp
c. 1–3 kb
d. More than 3 kb
e. 5–20 bp

Ans. b. 2–6 bp *(Ref: Robbins 9/e page 179)*

Repeat length Polymorphism or Short Tandem Repeats or Variable Number Tandem Repeats

Short repetitive sequence in human DNA is called Repeat length Polymorphism.

Most common one is dinucleotide repeat involve AC to TG on other strand.

Depending on the repeat size it is divided into:

Microsatellite -- Repeat size of 2–6 bp.

Mini satellite - Repeat size of 15–70 bp.

11. True about polymorphism is: (PGI Dec 06)

a. Single locus → multiple normal alleles
b. Single locus → multiple abnormal alleles
c. Single phenotype: Single locus → multiple normal alleles
d. Single phenotype: Single locus → multiple abnormal alleles

Ans. a, c.

- Normal variations in nucleotide sequence which occur in at least 1% of population is Polymorphism
- so they are multiple normal alleles in same locus
- Polymorphism can be in introns or exons so can be same phenotype or different phenotype depending on the location.

12. Microsatellite sequence is: (AI 2006)

a. Small satellite
b. Extra chromosomal DNA
c. Short sequence (2–5) repeat DNA
d. Looped-DNA

Ans. c. Short sequence (2–5) repeat DNA *(Ref: Robbins 9/e page 179)*

Repeat length Polymorphism or Short Tandem Repeats or Variable Number Tandem Repeats

Depending on the repeat size it is divided into:

Microsatellite -- Repeat size of 2–6 bp.

Mini satellite - Repeat size of 15–70 bp.

Epigenetics

13. Genes in CpG Island is inactivated by: (PGI Nov 2013)

a. Methylation
b. Metrylation
c. Ubiquitization
d. Acetylation

Ans. a. Methylation *(Robbins 9/e page 3-5)*

Gene promoters and regulation by methylation-demethylations

- Majority of gene promoters have high CG content.
- CpG islands are the common sites of methylation-demethylations
- Hence methylation-demethylations plays an important role in regulation of gene expression
- CpG islands of promoters are typically unmethylated, it favors transcription.

14. All are true DNA methylation except: (PGI Nov 2014)

a. It usually occurs in the cytosine
b. Can alter the gene expression pattern in cells

c. Role in genomic imprinting
d. No role in carcinogenesis
e. Essential for normal development

Ans. d. No role in carcinogenesis

(Robbins 9/e page 3-5)

Biochemical functions of Epigenetic ModificationQ

1. Regulation of tissue specific gene expression
2. X chromosome inactivation (Facultative Heterochromatin) one of the two X-chromosomes in every cell of a female
3. Genomic imprinting:
   Gene inactivation on selected chromosomal regions of autosomes is called Genomic Imprinting. This is the cause of preferential expression of one of the parental allele.
   Maternal imprinting refers to transcriptional silencing of the maternal allele.
   Paternal imprinting implies that the paternal allele is inactivated.
   Imprinting occurs in the ovum or the sperm, before fertilization.
4. Aging Process.

Epigenetic Changes Causing Pathological Alteration

1. Fragile X syndrome
   – Promoter site Hypermethylation causing FMR-1 gene silencing.
2. Cancer
   – *DNA methylation and histone modifications* dictate which genes are expressed, which in turn determines the lineage commitment and differentiation state of both normal and neoplastic cells.
   – Local promoter hypermethylation of tumor Suppressor gene leads decreased expression of the tumor suppressor gene.
3. DNA methylation is considered as a defence mechanism that minimise the expression of retroviral incorporated sequences.

15. All are true regarding epigenetics mechanisms except: (PGI may 2014)

a. Noninheritable
b. Acetylation of histone
c. Hereditary
d. Methylation of DNA
e. X chromosome inactivation

Ans. a. Noninheritable *(Ref: Harper 30/e page 440)*

Reversible heritable chemical modification^{Q} of DNA or histone or nonhistone proteins that does not alter DNA sequence itself is called epigenetics.

16. Random inactivation of X chromosome is

a. Lyonization
b. Allelic exclusion
c. Randomization
d. Genomic imprinting

Ans. a. Lyonization

Two factors that are peculiar to the sex chromosomes: (1) lyonization or inactivation of all but one X chromosome and (2) the modest amount of genetic material carried by the Y chromosome.

In 1961, Lyon outlined the idea of X-inactivation, now commonly known as the Lyon hypothesis. It states that (1) only one of the X chromosomes is genetically active, (2) the other X of either maternal or paternal origin undergoes heteropyknosis and is rendered inactive, (3) inactivation of either the maternal or paternal X occurs at random among all the cells of the blastocyst on or about day 16 of embryonic life, and (4) inactivation of the same X chromosome persists in all the cells derived from each precursor cell.

The inactive X can be seen in the interphase nucleus as a darkly staining small mass in contact with the nuclear membrane known as the Barr body, or X chromatin. The molecular basis of X inactivation involves a unique gene called XIST, whose product is a noncoding RNA that is retained in the nucleus, where it "coats" the X chromosome that it is transcribed from and initiates a gene-silencing process by chromatin modification and DNA methylation. The XIST allele is switched off in the active X.

True about 'X' chromosome inactivation:

a. XIST gene *(PGI Dec 06)*
b. RNA interference
c. Seen in male
d. Seen in female

Ans. a, b, d. XIST gene, RNA interference, Seen in female.

17. Histone acetylation cause: (AIIMS May 2011)

a. Increased heterochromatin formation
b. Increased ruchromatin formation
c. Methylation of cystine
d. DNA replication

Ans. b. Increased euchromatin formation

(Ref: Harrison 19/e page 421)

Euchromatin is transcriptionally active

Heterochromatin is transcriptionally inactive.

According to Harrison

Covalent posttranslational modifications of histones and other proteins play an important role in altering chromatin structure and, hence, transcription.

Histones can be reversibly modified in their amino-terminal tails, which protrude from the nucleosome core particle, by acetylation of lysine, phosphorylation of serine, or methylation of lysine and arginine residues.

Acetylation of histones by histone acetylases (HATs), for example, leads to unwinding of chromatin and accessibility to transcription factors.

Conversely, deacetylation by histone deacetylases (HDACs) results in a compact chromatin structure and silencing of transcription.

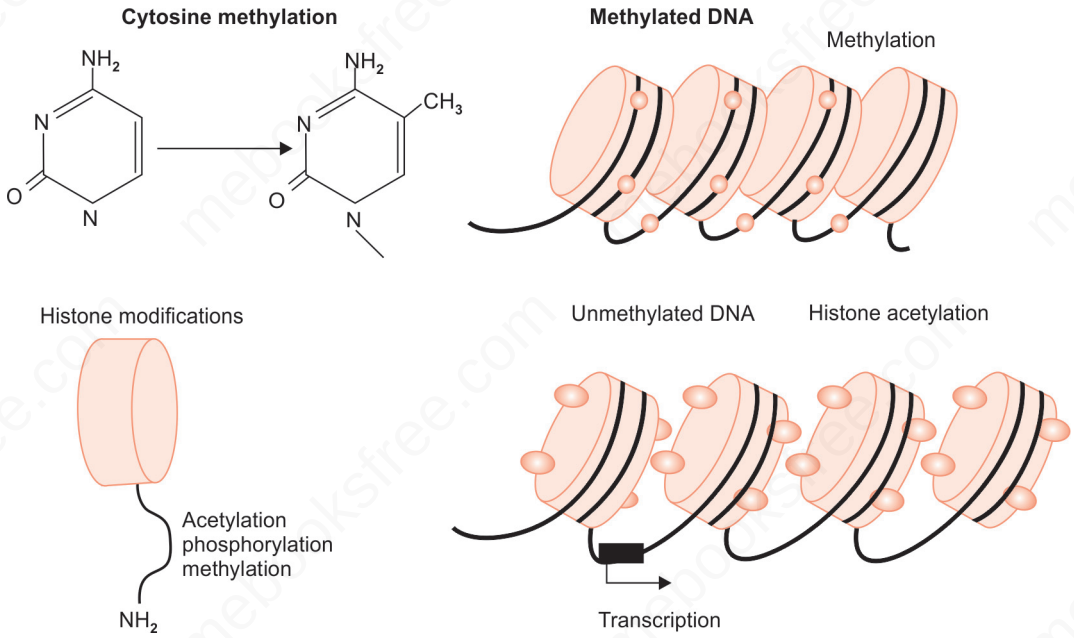


Fig. 13.11: Epigenetic modifications

18. Differential expression of same gene depending on parent of origin is referred to as: (AI 08)

a. Genomic imprinting
b. Mosaicism
c. Anticipation
d. Nonpenetrance

Ans. a. Genomic imprinting *(Ref: Robbins 9/e page 172)*

Studies over the past two decades have provided definite evidence that, at least with respect to some genes, important functional differences exist between the paternal allele and the maternal allele. These differences result from an epigenetic process called *imprinting*.

19. True about DNA methylation (PGI Nov 2010)

a. Alteration gene expression
b. Genetic code remains intact
c. Role in carcinogenesis
d. Protective mechanism against cleaving by restriction endonuclease

Ans. a, b, c, d. Alteration in gene expression, Genetic code remains intact, Role in carcinogenesis, Protective mechanism *(Ref: Harper 30/e page 440)*

Epigenetics

Reversible heritable chemical modification of DNA or histone or nonhistone proteins that does not alter DNA sequence itself.

The term epigenetics means, "above genetics" as the nucleotide sequence is unaltered.

This is one of the recently discovered method of regulation of gene expression.

This includes:

- DNA Methylation at Cytosine residues of CpG islands (Some consider this as a post-replicational modification)
- Post-translational modification of Histones.

20. Epigenetics is a: (NBE Pattern Question)

a. Chemical modification of DNA

b. Irreversible modification of DNA
c. Change in nucleotide sequence
d. Normal variation of nucleotides

Ans. a. Chemical modification of DNA

(Ref: Harper 30/e page 440)

The term "epigenetics" means "above genetics" and refers to the fact that these regulatory mechanisms do not change the underlying, regulated DNA sequence, but rather simply the expression patterns of this DNA.

Epigenetics refers to reversible, heritable changes in gene expression that occur without mutation. Such changes involve post-translational modifications of histones and DNA methylation, both of which affect gene expression.

21. Methylation of Cytidine residues of DNA will cause: (AIIMS 2014 May)

a. No Change
b. Decrease gene expression
c. Mutation
d. Increase in gene expression

Ans. b. Decrease in gene expression

(Ref: Harper 30/e page 440)

- DNA Methylation generally cause decrease in gene expression.

MiRNA and SiRNA

22. Function of miRNA is/are: (PGI May 2014)

a. Gene silencing
b. Gene activation
c. Transcription inhibition
d. Translation arrest
e. Cleavage of messenger RNA

Ans. a, d, e. Gene silencing, Translation arrest, Cleavage of messenger RNA. *(Ref: Harper page 447)*

miRNA and siRNA

Small noncoding single stranded RNAs which are 21-22 nucleotide length.

Main function- Post-transcriptional regulation of gene expression by altering mRNA function.

The regulation gene expression by miRNA

By altering mRNA function.

In 2006 Craig Mello and Andrew Fire were awarded Nobel Prize for silencing gene expression by miRNA

Steps involved are:

Binding of the miRNA to the target mRNA

If the miRNA-mRNA base pairing has one or more mismatches, translation of the cognate, target mRNA' is inhibited (**TRANSLATIONAL ARREST**)

- If the miRNA-mRNA base pairing is perfect over all 22 nucleotide
- The corresponding mRNA is degraded inside cytoplasmic organelle called **P Bodies**. (**mRNA DEGRADATION**)
- Functional Consequence of Translation Arrest and mRNA degradation by miRNA is **Silencing the gene expression or Gene Silencing**
- This is otherwise called RNA Interference

23. Normal role of Micro RNA is: (AI 2009)

a. Gene regulation
b. RNA splicing
c. Initiation of Translation
d. DNA conformation change

Ans. a. Gene regulation *(Ref: Harper 30/e page 447)*

Mutation

24. Which of the following is/are most severe/ dangerous change in gene: (PGI May 2014)

a. Deletion
b. Insertion
c. Mutation
d. Translocation
e. Duplication

Ans. a, b. Deletion, Insertion

Deletion and insertion causes garbling of reading frame. Hence, it is dangerous change in the polypeptide synthesized.

25. No loss of genetic material occur in: (AIIMS Nov 2012)

a. Deletion
b. Insertion
c. Substitution
d. Inversion

Ans. d. Inversion *(Ref: Robbins 9/e page 160)*

Structural Anomalies in Chromosome

1. Deletion
   Refers to loss of a portion of a chromosome.
   Most deletions are interstitial,
   Rarely terminal deletions may occur.
2. Ring chromosome
   Is a special form of deletion.

It is produced when a break occurs at both ends of a chromosome with fusion of the damaged ends.

Ring chromosomes do not behave normally in meiosis or mitosis and usually result in serious consequences

3. Inversion

Refers to a rearrangement that involves two breaks within a single chromosome with reincorporation of the inverted, intervening segment.

An inversion involving only one arm of the chromosome is known as *paracentric*.

If the breaks are on opposite sides of the centromere, it is known as *pericentric*.

Inversions are often fully compatible with normal development.

No loss of genetic element.

4. Isochromosome

Break along the axis perpendicular to the axis of chromosome.

One arm of a chromosome is lost

The remaining arm is duplicated, resulting in a chromosome consisting of two short arms only or of two long arms.

Loss of genetic element.

5. Translocation

A segment of one chromosome is transferred to another chromosome.

Balanced reciprocal translocation

- There are single breaks in each of two chromosomes, with exchange of material
- A balanced translocation carrier, however, is at increased risk for producing abnormal gametes
- No loss of genetic element

Robertsonian translocation (or centric fusion)

- A translocation between two acrocentric chromosomes
- Typically the breaks occur close to the centromeres of each chromosome
- Transfer of the segments then leads to one very large chromosome and one extremely small one. Usually the small product is lost.

26. True about Fragile X syndrome (PGI June 2009)

a. Trinucleotide repeat sequence disease
b. Chromosome-breakage
c. X-chromosome defect
d. Point-mutation
e. Deletion

Ans. a, c. Trinucleotide repeat sequence disease, X-chromosome defect.

27. Base substitution mutations can have the following molecular consequence except: (AI 2006)

a. Changes one codon for an amino acid into another codon for that same amino acid
b. Codon for one amino acid is changed into a codon of another amino acid
c. Reading frame changes downstream to the mutant site
d. Codon for one amino acid is changed into a translation termination codon

Ans. c. Reading frame changes downstream to the mutant site. *(Ref: Harper 30/e page 417)*

- Changes one codon for an amino acid into another codon for that same amino acid—Silent mutation
- Codon for one amino acid is changed into a codon of another amino acid—Missense mutation
- Codon for one amino acid is changed into a translation termination codon—Non-sense mutation
- Reading frame changes downstream to the mutant site—Frameshift mutation.

28. Frame shift mutation is caused by: (Ker 2007)

a. Deletion
b. Point mutation
c. Substitution
d. Transversion

Ans. a. Deletion *(Ref: Harper 30/e page 417)*

Frameshift Mutation

Due to insertion or deletion of nucleotides that are not a multiple of three results in frameshift mutation.

Reading frame is garbled.

29. Cystic fibrosis mutation causing the reduced chloride conductance is: (NBE pattern Q)

a. Class-1
b. Class-2
c. Class-3
d. Class-4

Ans. d. Class-4

Class IV: *Decreased conductance*. These mutations typically occur in the **transmembrane domain of** CFTR, which forms the ionic pore for chloride transport. There is a normal amount of CFTR at the apical membrane, but

with reduced function. This class is usually associated with a milder phenotype.

30. X-ray causes DNA mutation by: (PGI May 2014)

a. Double strand break repair
b. Oxidation
c. Pyrimidine Dimer
d. Intrastrand cross links

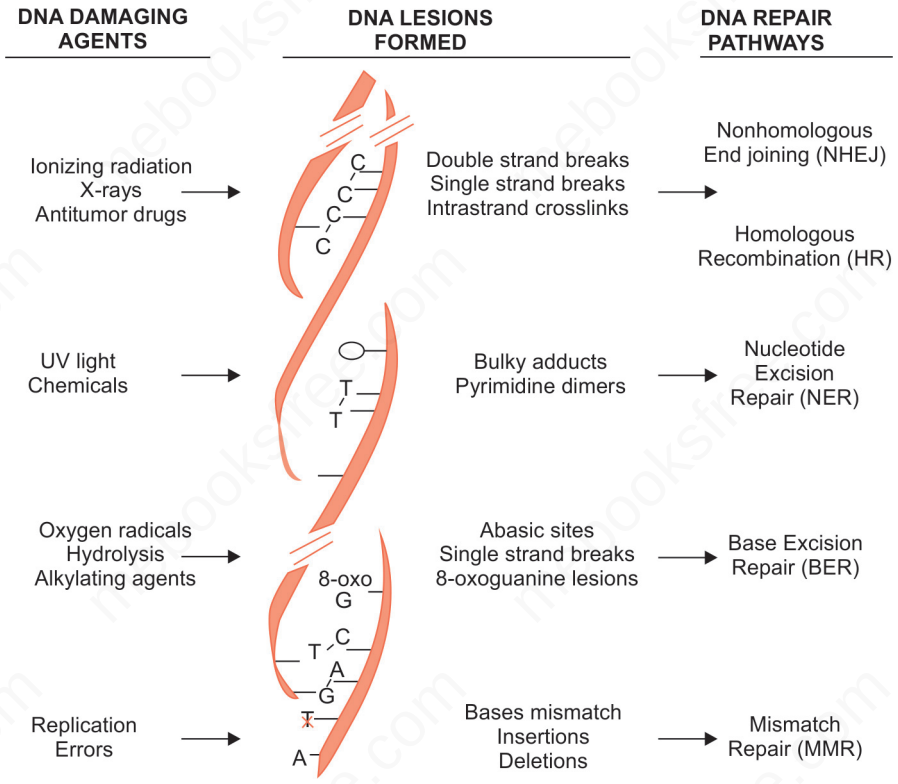
Ans. a, d. Double strand break repair, Intrastrand cross links.

31. One of the following mutations is potentially lethal: (Delhi 96)

a. Substitution of adenine for cytosine
b. Substitution of methylcytosine for cytosine
c. Substitution of guanine for cytosine
d. Insertion of one base

Ans. d. Insertion of base

Insertion or deletion of base can garble the reading frame, resulting in a frame shift mutation.



32. Sickle cell anemia is the clinical manifestation of homozygous genes for an abnormal hemoglobin molecule. The event responsible for the mutation in the B chain is: (AIIMS 91, Kerala 90)

a. Insertion
b. Deletion
c. Nondisjunction
d. Point mutation

Ans. d. Point mutation

The mutation in HbS is an example of:

- Point mutation
- Partially acceptable missense mutation
- Transversion
- Base substitution
- Nonconservative mutation.

33. Null mutation is: (AI 2000)

a. Mutation occurring in noncoding region
b. Mutation that does not change the amino acid or end product
c. Mutation that codes for a change in progeny without a chromosomal change
d. Mutation that leads to no functional gene product

Ans. d. Mutation that leads to no functional gene product

Null mutation

A mutation that leads to no functional gene product is called null mutation.

34. A mutation in the codon which causes a change in the coded amino acid, is known as: **(AIIMS May 02)**

a. Mitogenesis
b. Somatic mutation
c. Missense mutation
d. Recombination

Ans. c. Missense mutation *(Ref: Harper 30/e page 411)*

I. Silent Mutation
- If a mutation does not alter the polypeptide product of the gene.
- Also called Synonymous mutation.

II. Missense mutation
The alteration in the nucleotide may result in the incorporation of a different amino acid.

35. In a mutation if valine is replaced by which of the following would not result in any change in the function of protein: **(AIIMS May 02)**

a. Proline
b. Leucine
c. Glycine
d. Aspartic acid

Ans. b. Leucine

Valine and Leucine are branched chain nonpolar amino acids.

So homologous substitution.

36. Which of the following can be a homologous substitution for valine in the hemoglobin? **(AI 2004)**

a. Isoleucine
b. Glutamic acid
c. Phenyl alanine
d. Lysine

Ans. a. Isoleucine

Both are branched chain nonpolar amino acids.

37. Pyrimidine dimers are seen in:

a. UV rays
b. Xeroderma pigmentosa
c. Alkylating agents
d. X-rays

Ans. b. Xeroderma pigmentosa

(Ref: Harper 30/e page 390)

- DNA damaging agent that results in pyrimidine dimer is U-V light chemicals
- DNA repair mechanism that repair pyrimidine dimer is Nucleotide Excision repair
- Defective Nucleotide excision repair mechanism causes Xeroderma pigmentosum, Cockayne Syndrome, Trichothiodystrophy
- So pyrimidine dimers are seen in disorders caused by defective nucleotide excision repair.

14 Molecular Biology Techniques and Recent Advances in Molecular Biology

Topics Included

- Recombinant DNA Technology
- Gene Library
- Probes
- Amplification Techniques
- Hybridization and Blot Techniques
- Cytogenetic Techniques
- Mutation Detection Techniques
- DNA Sequencing Techniques
- Next Generation Sequencing
- Transgenic Animals
- Hybridoma Technique
- RFLP
- Stem Cell Biology
- Gene Therapy
- Human Genome Project
- Omics of Molecular Biology
- Bioinformatic and Genomic Sources
- Techniques to Study Proteomics
- Linkage and Association Studies

RECOMBINANT DNA TECHNOLOGY

Definition

In vivo amplification technique used to get a clone of desired DNA fragment.

To learn about Recombinant DNA Technology we should have knowledge about Vector, Restriction endonuclease, Chimeric DNA.

Restriction Endonuclease

- Endonuclease which cut the DNA at specific palindromic sequence.
- Palindrome is a sequence of duplex DNA that is the same when the two strands are read in opposite directions.

Examples: GATCC and CCTAG
AATT and TTAA

- They are otherwise called molecular scissors
- They cut the DNA to produce sticky or staggered ends or blunt ends
- They are called Restriction endonucleases because they restrict the entry of phages into host DNA.

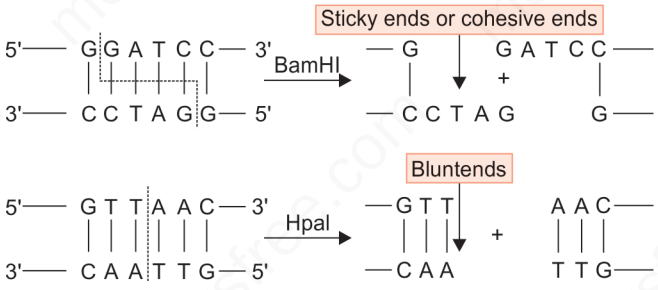


Fig. 14.1: Restriction endonucleases

Restriction Map

When a genomic DNA is treated with a specific Restriction endonuclease it cuts the DNA at specific sites to create a characteristic linear array of DNA. This is called restriction map.

Vectors used in Recombinant DNA Technology

Plasmids

- **Circular, double stranded** DNA[Q] molecules seen in bacteria (8-10 copies/cell).
- Extra chromosomal

- Each plasmid contains an origin of replication and can replicate independently
- They are episomes, i.e. a genome above or outside the bacterium
- Natural function is to confer antibiotic resistance
- Carry 0.01-10 kbp of DNA.

Phages (Bacterial Viruses)

- Virus which infect the bacteria are called phages.
- They have a linear DNA molecule
- Carry DNA fragments up to 10–20 kbp.

Cosmids

- They are plasmids which combine the features of Plasmid and Phages
- They contain special genes called cos site (needed for packing lambda DNA into phage particles)
- Carry DNA fragments up to 30–50 kbp.

BAC, YAC and PAC

Are artificially created chromosomes that can carry large DNA insert:

- BAC—Bacterial Artificial Chromosome
- YAC—Yeast Artificial Chromosome
- PAC—*E coli* bacteriophage P1-based vectors

DNA Insert Size:

BAC and PAC ---- 50–250 kbp

YAC ---- 500–3000 kbp

Chimeric DNA or Recombinant DNA

DNA to be cloned + Vector DNA = Chimeric DNA

Passenger DNA

DNA to be cloned is called Passenger DNA or Foreign DNA.

Procedure to Prepare Chimeric DNA

- Both the foreign DNA and vector DNA is treated with same Restriction endonuclease
- This creates sticky or blunt ends
- This is religated using DNA Ligase
- Thus, chimeric DNA is produced.

Homopolymer Tailing

Technique used to overcome the problems inherent to sticky ends and blunt ends.

1. **Problems with Sticky End**
   - Sticky ends of a vector may reconnect with themselves, with no net gain of DNA
   - Sticky ends of fragments also anneal so that heterogeneous tandem inserts form
   - Sticky-end sites may not be available or in a convenient position for the restriction endonuclease
2. **Problems with Blunt Ends**
   - Blunt ends ligation is not directional.

Remember

The enzyme that helps in blunt end ligation is Bacteriophage T4 Enzyme DNA Ligase.

Procedure of Homopolymer Tailing

- To circumvent the problems of blunt ends, homopolymer tailing is used
- New synthetic sticky ends are added using the enzyme **terminal transferase**
- Poly d(G) is added to the 3′ ends of the vector
- Poly d(C) is added to the 3′ ends of the foreign DNA using terminal transferase
- Then the two molecules can only anneal to each other This procedure is called homopolymer tailing.

Blue-white Screening

- Is a quick and easy screening technique to detect successful ligation of DNA of interest to the vector
- Cells are grown in the presence of **X-Galactose**
- If the ligation **was successful**, the bacterial colony will be **white**
- If not, the colony will be **blue**.

Recombinases–An Adjunct to Restriction Endonucleases

Catalyse specific incorporation of two DNA fragments that carry the appropriate recognition sequences and carry out homologous recombination, between relevant recognition site.

Examples of Recombinases and relevant recognition site

Recombinase	Recognition site
CRE Recombinase	Bacterial Lox P site
λ phage encoded INT protein	Bacteriphage λ TT site
Yeast FLP Recombinases	Yeast FRT site

Steps of Recombinant DNA Technology

- Isolation of specific DNA
- Selection of Vector
- Synthesis of chimeric DNA
- Introduction of recombinant plasmid to bacteria
- Screening for Recombinant Vectors
- Selection of Specific DNA clones.

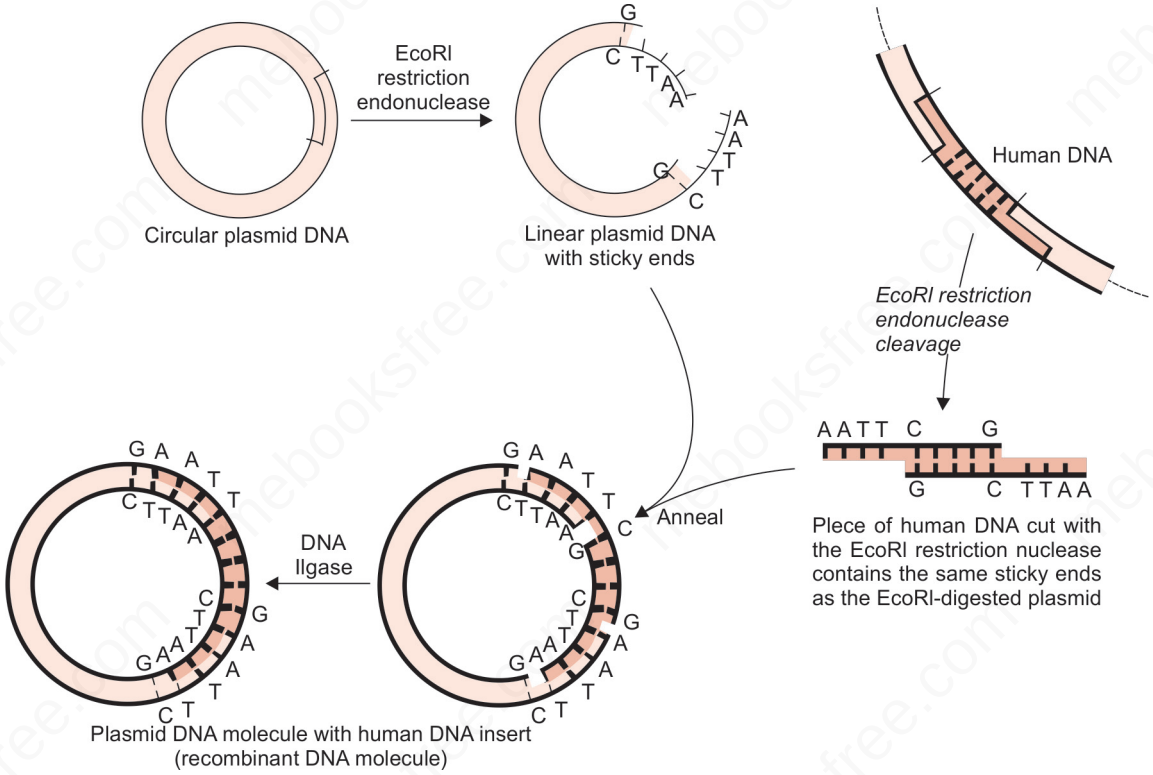


Fig. 14.2: Steps of recombinant DNA technology

Uses of Recombinant DNA Technology in Clinical Medicine

- To understand molecular basis of diseases
- Preparation of vaccines and hormones
- Diagnosis of infectious diseases
- Forensic medicine—reveal a criminal from specimens left on the scene of crime.

CRISPR cas 9 System

- Clustered Regularly Interspersed Short Palindromic Repeats associated gene 9.
- A prokaryotic 'immune system' conferring resistance to external genes from bacteriophage.

Mechanism of action

In bacteria

CRISPR derived RNA combines with the Cas nuclease to target and specifically cleave the DNA of invading phage, thereby inactivating these invading genomes and protecting the bacterium from productive phage infection and lysis

In eukaryotes

- This system now emerged as Novel DNA/genome editing or gene regulatory system
- CRISPR use an RNA based targeting to bring Cas 9 nuclease to foreign DNA.

Contd ...

Contd ...

- This CRISPR-RNA Cas 9 complex then inactivates and degrade target DNA.
- This system can be used in eukaryotic cells, including humans.

Application of CRISPR Cas 9

- Genomic editing
- Targeted Mutagenesis—Gene knock out
- Modulation of gene expression
- Gene deletion.

cDNA[Q]

DNA complementary to mRNA is called cDNA or Copy DNA or Complementary DNA.

Procedure to Prepare cDNA

- Isolate the mRNA
- By the action of Reverse Transcriptase RNA-DNA hybrid is synthesized
- By RNAseH RNA is digested
- By DNA Polymerase double stranded DNA is produced
- Thus, DNA complementary to mRNA is synthesized.

Gene Library

A collection of recombinant DNA clones generated from a specific source.

Two Types of Gene Library

1. **Genomic DNA Library:**
   - Prepared from total genomic DNA of an organism
   - By digestion of Genomic DNA by Restriction Endonuclease
   - Then recombinant clones of such digested DNA is produced by recombinant DNA Technology.
2. **c DNA library**
   - cDNA is prepared from mRNA by the action of Reverse Transcriptase
   - The recombinant clones for cDNA are produced by Recombinant DNA Technology.

Advantages of cDNA over Genomic DNAQ

- Contains only coding sequences
- Represent the mRNA in a tissue
- Hence used to study gene expression.

Probes

Pieces of DNA or RNA labelled by various techniques to detect a complementary sequence.

Uses of Probes

- To detect DNA on Southern blot transfers
- To detect RNA on Northern blot transfers
- Can search libraries for a specific gene.

Labelling of DNA Probes

Two types of labels

Radioactive Labels

- Most commonly by Radioactive Phosphorus (^{32}P)

Nonradioactive Labels

- Biotin label
- Fluorescent labels

Methods of Radiolabelling

1. End labelling at 5′ end or 3′ end of the probe.
2. Nick translation.

END LABELLING OF PROBES

At 5′ end

Enzymes used are:

- Alkaline Phosphatase
- Polynucleotide Kinase

At 3′ end

Enzyme used is:

- Terminal Transferase

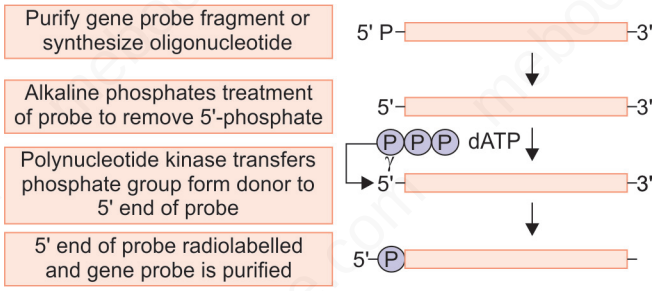


Fig. 14.3: End labelling at 5' end

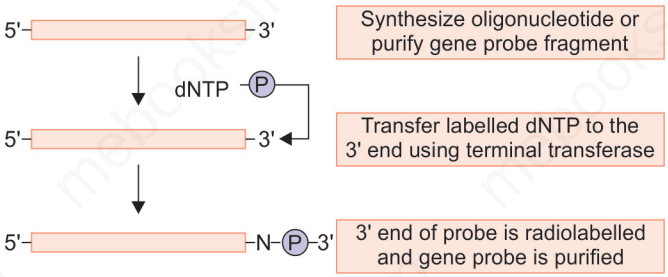


Fig. 14.4: End labelling at 3' end

Nick Translation

The technique used to produce radiactive labelled DNA probes.

Procedure

- Single stranded nick created and the dNucleotide is removed by DNAse I
- Gap is filled by radiolabelled dNucleotide by DNA Polymerase
- Thus, radiolabelled DNA probe is created.

Expression Vector

A vector in which the foreign DNA introduced by Recombinant DNA Technology synthesizes protein, i.e. the gene is expressed.

Uses of Expression Vectors

- To detect specific cDNA
- To detect specific protein produced by a specific cDNA
- To produce proteins like Insulin in large quantities.

Enzymes used in Recombinant DNA Research

Enzyme	Reaction	Uses
Alkaline phosphatase	Dephosphorylates 5' ends of RNA and DNA	Removal of 5'-PO_4 groups prior to kinase labeling; also used to prevent self-ligation
DNA ligase	Catalyzes bonds between DNA molecules	Joining of DNA molecules

Contd...

Contd...

Enzyme	Reaction	Uses
DNA polymerase I	Synthesizes double-stranded DNA from single-stranded DNA	Synthesis of double-stranded cDNA; Nick translation; Generation of blunt ends from sticky ends
Thermostable DNA polymerases (Taq Polymerase)	Synthesize DNA at elevated temperatures (60–80°C)	Polymerase chain reaction (DNA synthesis)
DNase I	Under appropriate conditions, produces single-stranded nicks in DNA	Nick translation Mapping of hypersensitive sites Mapping protein-DNA interactions
Exonuclease III	Removes nucleotides from 3' ends of DNA	DNA sequencing Mapping of DNA-protein interactions
λ Exonuclease	Removes nucleotides from 5' ends of DNA	DNA sequencing
Polynucleotide kinase	Transfers terminal phosphate (ϒ position) from ATP to 5'-OH groups of DNA or RNA	^{32}P end-labeling of DNA or RNA
Reverse transcriptase	Synthesizes DNA from RNA template	Synthesis of cDNA from mRNA; RNA (5' end) mapping studies
S1 nuclease	Degrades single-stranded DNA	Removal of 'hairpin' in synthesis of cDNA; RNA mapping studies (both 5' and 3' ends)
Terminal transferase	Adds nucleotides to the 3' ends of DNA	Homopolymer tailing
CRISPR-Cas9	RNA targeted DNA directed Nuclease	Genome deiting and modulation of gene expression

AMPLIFICATION TECHNIQUES

Polymerase Chain Reaction

- Revolutionary technique invented by Karry B Mullis[Q] in 1989
- He got Nobel Prize for this in 1993
- The polymerase chain reaction (PCR) is a test tube method for amplifying a selected DNA sequence
- Exponential[Q] amplification of the sample
- The number of samples after n number of cycles is 2^n
- One cycle of PCR require <5 to 10 minutes
- 20 cycles result in million-fold amplification of the target DNA
- Product obtained by amplification is called Amplicon.

The instrument that takes samples through the multiple steps of changing temperature in PCR Cycle is called Thermocycler.

Steps of PCR Cycle[Q]

1. **Denature the DNA:** The DNA to be amplified is heated to separate the double-stranded target DNA into single strands 94°C.
2. **Annealing of primers to ssDNA:** The separated strands are cooled and allowed to anneal to the two primers (one for each strand). By cooling to 55°C.
3. **Extension of the Primer:** Synthesize new chains complementary to the original DNA chains. By heating again to 72°C.

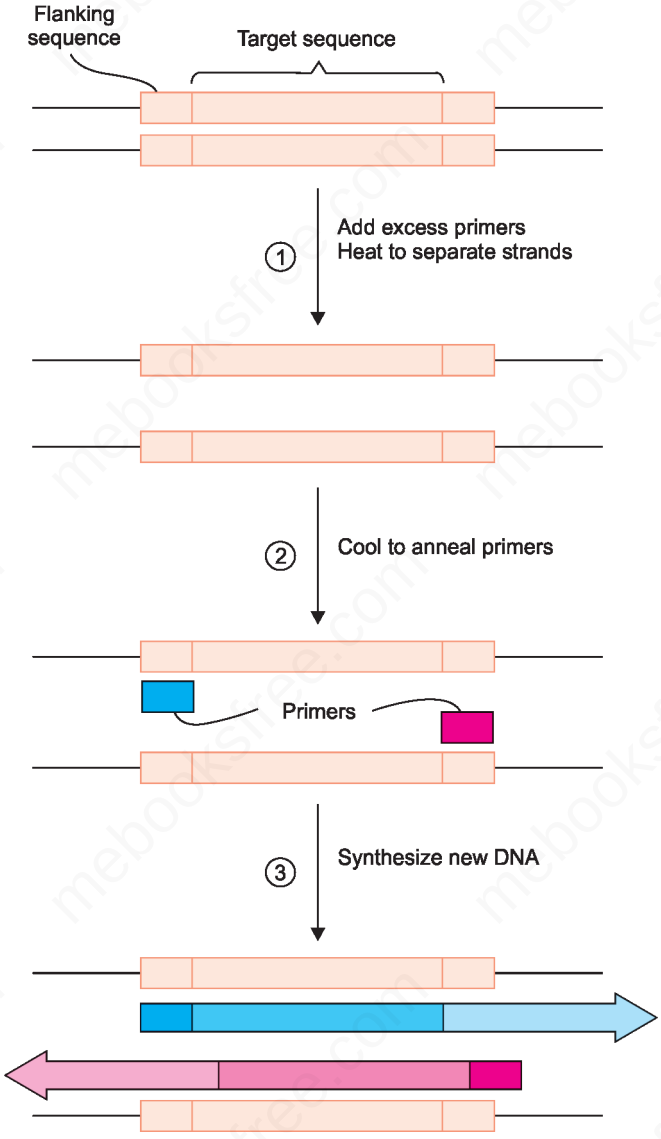


Fig. 14.5: The PCR cycle

Pre-requisites of PCR

- Sample DNA to be amplified
- Deoxy nucleotides
- Thermostable Polymerase-Taq Polymerase[Q] obtained from Thermus Aquaticus found in hot springs.
- Primer
- MgCl2 and KCl.

Phusion

- Newly developed DNA Polymerase for PCR
- High fidelity DNA Polymerase
- Enzyme constructed by fusion of unique DNA binding domain to Pyrococcus like Proofreading Polymerase
- High Processivity
- Low error rate
- Not inhibited by any quantity of blood, where as Taq Polymerase is inhibited.

Variants of PCR

1. **Reverse Transcriptase PCR (RT PCR)**
   - It is the PCR amplification of a reverse transcription product
   - **RT PCR** amplifies very small amounts of any kinds of RNA **(mRNA, rRNA, tRNA etc.)**
   - cDNA copies of mRNA generated by a retroviral reverse transcriptase
   - This cDNA is amplified as in usual PCR
   - TthPolymerase (from Thermus thermophilus) is used
   - Method used to obtain relative expression of gene in a cell.
2. **Real time (Homogenous or Kinetic) PCR**
   - This is a type of Quantitative PCR
   - Methods used to amplify and at the same time quantitate amplified PCR products during the exponential phase of PCR cycle (called Realtime). It is not after the amplification is over.

Methods used to quantitate the number of copies DNA present within the genome.

Intercalating Dyes:

- Ethidium Bromide
- SYBR Green.[Q]

Sequence Specific Probes:

- TaqMan
- Molecular beacon
- **Fret Probes:** Fluorescence Resonance Energy Transfer Probes.

3. **Nested PCR**

   When the DNA to be amplified is present in low concentration compared to total DNA in the sample, this technique is used

   Nested PCR uses two sets of amplification primers.
   - Outer primers
   - Inner primers.
4. **Multiplex PCR**
   - Simultaneous amplification of many targets in one reaction
   - Uses more than one pair of primers.
5. **Arbitrary PCR (Arbitrarily Primed Polymerase Chain Reaction) (AP PCR)**

Main Features:

- Uses short random primers (Usually 10 bases)
- Amplifies anonymous stretches of DNA

Applications of PCR

1. Used in Forensic Medicine
2. To detect infectious agents, especially latent viruses
3. To make prenatal genetic diagnoses
4. To detect allelic polymorphisms
5. To detect mutation
6. To establish precise tissue types for transplants
7. To study evolution, using DNA from archeological samples
8. For quantitative RNA analysis after RNA copying and mRNA quantitation by the so-called Reverse Transcriptase PCR method
9. **To score *in vivo* protein:** DNA occupancy using chromatin immunoprecipitation assays (ChIP)
10. To facilitate New Generation Sequencing.

HYBRIDIZATION AND BLOT TECHNIQUES

1. **Southern Blot**
   - Devised by Edward Southern in 1975
   - Technique to detect **specific DNA Segment.**

   Principle:
   - Based on specific base pairing rule of complementary nucleic acid strands
   - It is a **DNA-DNA Hybridization**.

   Steps of Southern Blot
   - Duplex DNA isolated
   - Treated with Restriction Endonuclease
   - DNA is fragmented

- Fragmented DNA separated by Agar gel electrophoresis
- Treated with NaOH to denature the DNA
- Denatured DNA fragments transferred to Nitrocellulose membraneor nylon paper (Blot)
- Add Radiolabelled or Fluorescent cDNA probes
- Detection of Hybridization by imaging (DNA-DNA Hybridization).

Uses of Southern Blot

- Identification of specific viral or bacterial DNA in the infected sample
- Screening test to detect inborn errors
- Detect point mutations
- Can detect gene alteration, like deletion, insertion etc.
- In forensic medicine, to analyze DNAs from specimens at the scene of crime—blood, semen, saliva etc.

2. **Northern Blot**
   - Technique used to detect specific RNA
   - Principle: RNA- DNA hybridization technique
   - Radioactive/fluorescent labelled cDNA Probes used.

   Uses
   - Used to detect specific gene expression in specific tissues.

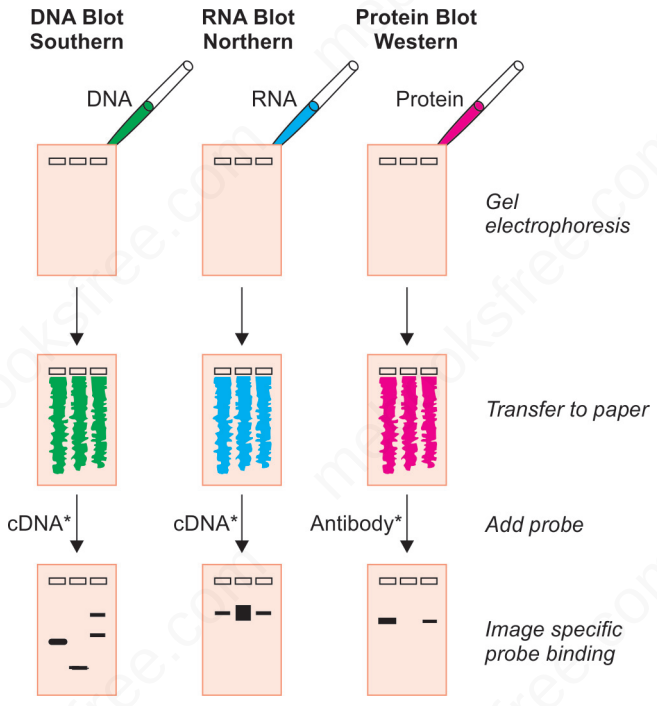


Fig. 14.6: Blot techniques

3. **Western Blot (Immunoblot) analysis for Proteins**
   Technique to detect specific Protein in a sample.

   Principle:
   - Antigen antibody Interaction
   - Radioactive labeled antibody used.

   Uses:
   - Identification of a specific protein in sample
   - Detection of Viral pathogens by identifying viral proteins—HIV virus or Hepatitis B virus.
4. **South Western Blotting**
   To examine Protein–DNA Interaction
5. **Dot blot Technique**
   - The step, blotting to nitrocellulose membrane is avoided
   - The sample is directly applied to slots on a specific blotting apparatus containing nylon membrane. This is also called *slot blot*.

CYTOGENETIC TECHNIQUES

The word chromosome is a combination of two words greek words, '**Chroma**' means '**color**' and '**Somes**' means '**body**'. So chromosomes are colored bodies.

Arms of Chromosome

- p arm is the short arm. p stands for petite
- q arm is the long arm. q is the next letter after p
- q arm is also called g arm. g stands for grande

Cytogenetic Techniques

Can be divided into:

- Conventional Cytogenetic techniques-Chromosome Banding
- Molecular Cytogenetic Techniques—Fluorescent in Situ Hybridization (FISH)
- Array Based Techniques (Cytogenomic techniques)
  - Comparative Genomic Hybridization (CGH) array
  - Single Nucleotide Polymorphism (SNP) array

Samples for chromosome analysis

- Prenatal (fetal) chromosome
- Amniocytes by Amniocentesis
- Chorionic villi by Chorionic Villus Sampling—Allows early detection of anomalies <10 weeks
- Fetal Blood by Percutaneous Umbilical cord sampling (PUBS) in late second trimester.

For preimplantation detection of anomalies

- Analysis of Blastomere

Other Samples in adults are:

1. Cultured skin fibroblast
2. Bone marrow
3. Peripheral lymphocyte

Timing for Chromosome Analysis

Artificially arrested in mitosis during Metaphase (or prometaphase)

- Metaphase arrest by - N deacetyl N-methyl Colchicine (Colcemid)

Conventional Cytogenetics—Banding Techniques

- Cytogenetic analysis is most commonly carried out on cells in mitosis, requiring dividing cells
- Halting mitosis in metaphase is essential, because chromosomes are at their most condensed state during this stage of mitosis
- The banding pattern of a metaphase chromosome is easily recognizable and is ideal for karyotyping.

Important Banding Techniques

G Banding

- Other name Gband Trypsin Giemsa (GTG) Banding
- The chromosomes are treated with Trypsin
- Stained by Giemsa
- Produce dark (G+) and light (G–)
- Visualized under light Microscope
- Most commonly used

R (Reverse) Banding

- Stained by Giemsa, Acridine Orange
- Yield light and dark band which are reverse of G Banding
- Visualized under light microscope
- Used for detecting rearrangements at the terminal end of chromosome (telomeres).

C Banding

- Centromeric Heterochromatin Banding
- Pretreated with acid followed by alkali
- Stained by Giemsa
- Visualized under light microscope
- Centromeric and Heterochromatic regions are preferentially stained
- To study Chromosomal translocation in the centromere.

Q Banding

- Stained by Quinacrine Mustard
- Visualized under Fluorescence Microscope
- Bands similar to G banding
- Fluorescent Bands are obtained.

Disadvantages of Conventional Banding Techniques

- Deletions smaller than several million base pairs are not routinely detectable by standard G-banding techniques
- Chromosomal abnormalities with indistinct or novel banding patterns can be difficult or impossible to interpret.
- To carry out cytogenetic analysis, cells must be dividing, which is not always possible to obtain (e.g. in autopsy or tumor material that has already been fixed).

MOLECULAR CYTOGENETIC TECHNIQUE—FLUORESCENT IN SITU HYBRIDIZATION (FISH)

Simple detection of specific genetic information in **a morphologically intact tissue**, cell or chromosome using fluorescent probes.

To metaphase spread of chromosomes on a glass slide fluorescent probe is added.

Advantages of FISH

- FISH permits determination of the number and location of specific DNA sequences in human cells
- FISH can be performed on metaphase chromosomes, as with G-banding, but can also be performed on cells not actively progressing through mitosis
- FISH performed on nondividing cells is referred to as interphase or nuclear FISH.

Disadvantages of FISH

- FISH requires a preselection of an informative molecular probe prior to analysis
- So a prior knowledge of the anomaly is needed.

Uses of FISH[Q]

- Detection of numeric abnormalities of chromosomes (aneuploidy)
- The demonstration of subtle microdeletions
- Detection of complex translocations not detectable by routine karyotyping
- For analysis of gene amplification
  e.g. HER2/NEU in breast cancer or N-MYC amplification in neuroblastomas
- For mapping newly isolated genes[Q] of interest to their chromosomal loci.

Chromosome Painting

- Is an extension of FISH
- Probes are prepared for entire chromosomes

- Different chromosomes are identified by different fluorescently labelled probes
- The number of chromosomes that can be *detected simultaneously* by chromosome painting is limited by the availability of fluorescent dyes.

Multicolor FISH or Spectral Karyotyping

- Similar to Chromosome painting in which probe is prepared for entire Chromosome
- But unlike chromosome painting, a combination of five fluorochromes are used
- 23 distinct mixtures of 5 fluorophores to create a unique 'color' for each chromosome
- By appropriate computer-generated signals, the entire human genome can be visualized.

ARRAY-BASED METHODOLOGIES (CYTOGENOMICS)

Array-based methods were introduced into the clinical lab beginning in 2003 and quickly revolutionized the field of cytogenetics They are:

1. CGH array
2. SNP array

Advantages of Array-based Techniques

- Permit analysis of many regions of the genome in a single analysis
- High resolution over standard cytogenetics.

Before discussing array CGH, lets learn about Microarray techniques

MICROARRAY TECHNIQUE[Q]

- Other names are **DNA Chip**
- DNA microarrays contain thousands of known immobilized DNA sequences organized in an area no larger than a microscope slide
- The fluorescently tagged sample to be sequenced is added.

DNA Microarray or DNA Chip

- To the DNA Chip containing known oligonucleotide sequence, fluorescently labelled unknown oligonucleotide is added
- The computerized algorithms can decode the unknown oligonucleotide, by detecting the location of fluorescent hybridization pattern on the chip
- This technique is used for genotyping or genome sequencing.

RNA Microarray

- To the known array of oligonucleotide, fluorescently labelled cDNA prepared from unknown mRNA is added
- Computerized algorithms can then rapidly 'decode' the cDNA sequence based on fluorescent hybridization pattern on the chip
- This technique is used for gene expression studies.

Protein microarray

- Immobilized known antibodies placed on the glass slide
- Fluorescently tagged target protein added
- By antigen: antibody interaction target protein detected
- This technique used in the study of proteomics.

Uses of microarrays[Q]

- To analyze a DNA sample for the presence of gene variations or mutations (genotyping)
- To determine the patterns of mRNA production (gene expression analysis)
- To study Proteins (Protein Microarray)
- To SNP genotyping.

Array-Based Comparative Genomic Hybridization (Array CGH)

- This is also a hybridization technique done on a Microarray or DNA Chip, hence in the name array – based
- Here two Genomes are compared, hence the name Comparative Genomic Hybridization.

Procedure

In array CGH the test DNA and a reference (normal) DNA are labelled with two different fluorescent dyes (most commonly Cy5 and Cy3, which fluoresce red and green, respectively).

The differentially labelled samples are added to a DNA Chip spotted with entire human genome (usually cover all 22 autosomes and the X chromosome) at regularly spaced intervals.

- If the contributions of both samples are equal for a given chromosomal region
  - Then all spots on the array will fluoresce yellow
  - The result of an equal admixture of green and red dyes.
- If the test sample shows an excess of DNA at any given chromosomal region (such as resulting from an amplification)

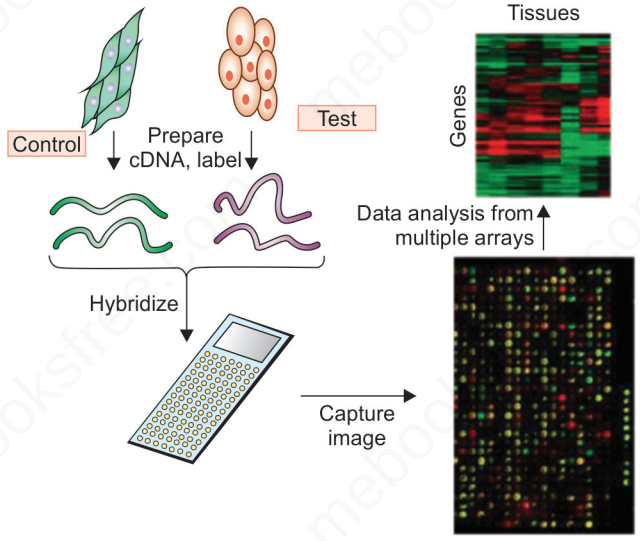


Fig. 14.7: Array CGH

- There will be a corresponding excess of signal from the dye with which this sample was labeled

- If the test sample show deletion,
  - There will be an excess of the signal used for labelling the reference sample (green).

Uses of Array CGH

1. Detect gene amplification
2. Detect gene deletion
3. Detect copy number variations.

Hence used diseases of unknown etiology like Cancer, Autism, Mental Retardation, Child with dysmorphic features etc.

Remember
Array CGH cannot detect Balanced Translocations

SNP Array

SNP platforms use arrays to find out SNPs that are distributed across the genome.

(Please see DNA Polymorphisms in Chapter Regulation of Gene expression, to know about SNPs)

Uses

- Used in genome-wide association studies to identify disease susceptibility genes
- To identify genomic deletions and duplications
- To detect regions of the genome that have an excess of homozygous genotypes and absence of heterozygous genotypes (e.g. CC and TT genotypes only, with no CT genotypes).

Comparison of Different Cytogenetic Techniques

Method	Requires growing cells	Detects deletion and duplication	Detects balanced structural rearrangements	Detects Uniparental Disomy	Lower limits of detection
G Banding	Yes	Yes	Yes	No	5–10 mb
Metaphase FISH	Yes	Yes	Yes	No	40–250 thousand mb
Interphase FISH	No	Yes	Some	No	40–250 thousand mb
CGH array	No	Yes	No	No	Single Exon or Single gene
SNP array	No	Yes	No	Some	Single Exon or Single gene

Methods to detect aneuploidy
- Conventional Karyotyping
- FISH
- Microarray based CGH
- Multiple Ligation dependent Probe Amplification (MLPA)
- Real time PCR
- Quantitative Fluorescent PCR (QF–PCR)
- Multiplex amplifiable probe hybridization (MAPH).

MUTATION DETECTION TECHNIQUES

Test to Detect Mutations

1. Ame's Test
   - Test to detect mutagenicity
   - Special strains of *Salmonella typhimurium* have mutated histidine gene
   - Hence, they will grow only in medium containing Histidine gene
   - This is called reverse mutation
   - The number of colonies is proportional to the quantity of mutagens.
2. Site Directed Mutagenesis
   - Michael Smith in 1993
   - An oligodeoxyribonucleotide whose sequence is complementary to a part of known gene is synthesized
   - A specific deletion/insertion is produced in the oligodeoxynucleotide

- It is then extended by DNAP
- This altered gene is amplified and expressed by insertion into a cloning vector
- This allows the study of a particular mutation.

Techniques used to Detect Mutations with DNA Sequence Alterations

I. First do a PCR amplification of the DNA, then different sequencing techniques can be used

- ***Sanger's technique***: 36 years after its Nobel–worthy invention by Frederick Sanger, Sanger sequencing is still considered the 'gold standard' for sequence determination
- ***Pyrosequencing***

Principle:

- When a nucleotide is incorporated into a growing DNA strand, there is release of pyrophosphate (PPi).
- In this technique, individual nucleotides (A, C, T, or G) one at a time into the reaction
- If one or more nucleotides are incorporated into the growing strand of DNA, pyrophosphate is released
- *Released Pyrophosphate participate in* a secondary reaction
- A secondary reaction involving luciferase that produces light, which is measured by a photo detector

Advantage of Pyrosequencing

- More sensitive than Sanger sequencing
- Allowing for detection of as little as 5% mutated alleles in a background of normal alleles
- To analyze DNA obtained from cancer biopsies, in which tumor cells are often, contaminated with large numbers of admixed stromal cells

II. Restriction Fragment length Polymorphism:

- If a mutation affect a specific restriction site, this technique can be used.

Techniques Used to Detect Mutations that Affect Length of DNA

a. Amplicon Length Analysis
b. Real-time PCR
c. Multiple Ligation Dependent Probe Amplification.

Amplicon length analysis

- After doing PCR, the size of PCR products determined by gel electrophoresis.

Real–time PCR

- Detect and quantify the presence of particular nucleic acid sequences in "real time" (i.e. during the exponential phase of DNA amplification rather than post–PCR).

Multiplex Ligation–Dependent Probe Amplification (MLPA)

- MLPA blends DNA hybridization, DNA ligation, and PCR amplification

Uses

- To detect deletions and duplications of any size, including anomalies that are too large to be detected by PCR and too small to be identified by FISH.

Procedure of MLPA

- Each MLPA reaction uses a pair of probes that can hybridize side–by–side to one strand of the target DNA.
- Once bound, the probes are covalently joined via a ligase reaction

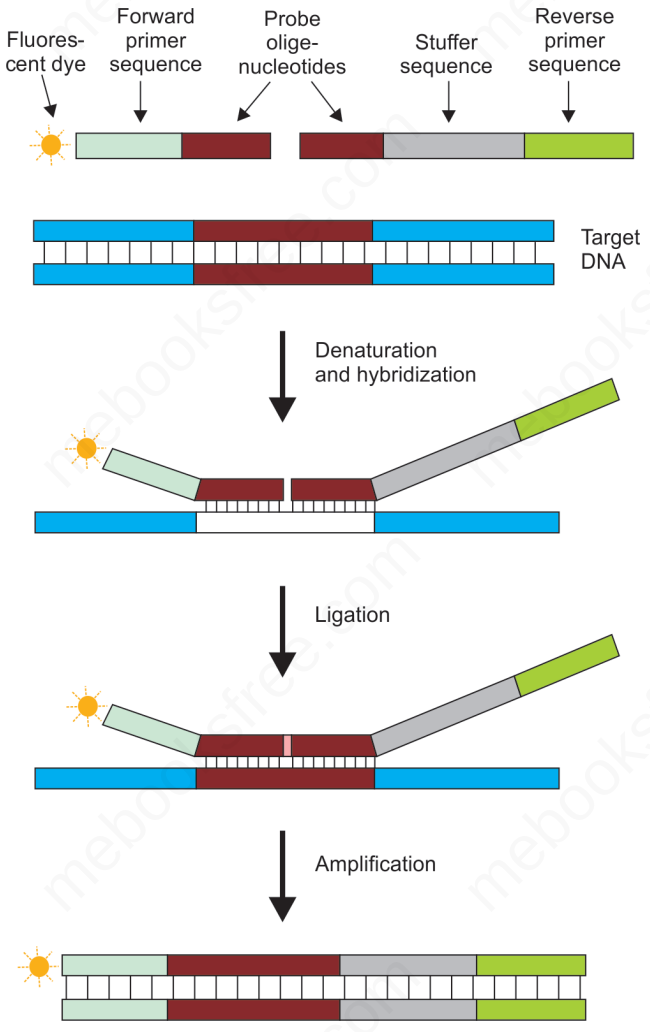


Fig. 14.8: Multiple ligation dependent probe amplification

- In addition to the target sequence, the probes also contain additional sequences at their ends that can be used as primer sequences in a PCR

- The ligated probes thus create a template that can then be amplified by PCR.

Advantages of MLPA

- Can be performed on very small amounts of genomic DNA
- Each probe–set can be designed with identical primer sequences
- Many probe–sets can be applied and amplified in one reaction tube.

Other Mutation Detection Technique[Q]

Method	Type of Mutation Detected
Cytogenetic analysis	Numerical or structural abnormalities in chromosomes
Fluorescent in situ hybridization (FISH)	Numerical or structural abnormalities in chromosomes
Southern blot	Large deletion, insertion, rearrangement, expansions of triplet repeat, amplification
Polymerase chain reaction (PCR)	Expansion of triplet repeats, variable number of tandem repeats (VNTR), gene rearrangements, translocations; prepare DNA for other mutation methods
Reverse transcriptase PCR (RT–PCR)	Analyze expressed mRNA (cDNA) sequence; detect loss of expression
DNA sequencing	Point mutations, small deletions and insertions
Restriction fragment polymorphism (RFLP)	Point mutations, small deletions and insertions
Single–strand conformational polymorphism (SSCP)	Point mutations, small deletions and insertions
Denaturing gradient gel electrophoresis (DGGE)	Point mutations, small deletions and insertions
RNAse cleavage	Point mutations, small deletions and insertions
Oligonucleotide specific hybridization (OSH)	Point mutations, small deletions and insertions
Microarrays	Point mutations, small deletions and insertions Genotyping of SNPs
Protein truncation test (PTT)	Mutations leading to premature truncations
Pyrosequencing	Sequencing of whole genomes of microorganisms, resequencing of amplicons
Multiplex ligation–dependent probe amplification (MLPA)	Copy number variations

DNA SEQUENCING TECHNIQUES

1. **Sanger's Technique (Controlled Chain Termination Method)**
   - Chain terminators used 2′ 3′ Dideoxynucleotides
   - DNA Polymerase used is Klenow Polymerase
   - This can be automated

Principle:

- Introduction of 2′ 3′ dideoxy nucleotides will terminate the DNA synthesis because no free 3′OH group for the formation of next phosphodiester bond.

2. **Maxam and Gilbert Chemical Cleavage Method**
3. **Next Generation Sequencing (NGS)**

Next Generation Sequencing (NGS)

Term used to describe several newer DNA sequencing technologies that are capable of producing large amounts of sequence data in a massively parallel manner.

Comparison of Sanger's Sequencing and Next Generation Sequencing

Sanger's Sequencing	New Generation Sequencing
High Cost	Low cost
Single, simple homogenous template DNA	No such requirement, sample from almost any source can be used
Provides an "average" result for a DNA sample. Uninterpretable results if sample of DNA is heterogenous	Well suited for heterogenous DNA Sample

Three Basic Processes of NGS

Spatial separation

At the beginning of the procedure, individual input DNA molecules are physically isolated from each other in space. The specifics of this process are platform–dependent.

Local amplification

After separation, the individual DNA molecules are amplified in situ using a limited number of PCR cycles.

Parallel sequencing

The amplified DNA molecules are simultaneously sequenced by the addition of polymerases and other reagents, with each spatially separated and amplified original molecule yielding a, "read" corresponding to its sequence.

Some New Sequencing Techniques

CAGE (Cap analysis of gene expression)

A method that allows the selective capture, amplification, cloning, and sequencing of mRNAs via the 5'Cap structure

RNA–Sequence

mRNAs are converted to cDNAs using reverse transcription, and these cDNAs are amplified and directly sequenced. This method is termed **RNA–Sequence**.

GRO–Sequence (Global Run–on Sequencing)

A method where nascent transcript are specifically captured and sequenced using NGS sequencing.

This helps to map location of active transcription complexes.

NET–Sequence (Native elongating transcript sequencing)

This technique allows for sequencing of RNA within elongating RNA polymerase–DNA–RNA ternary complexes. This helps in genome–wide analysis of transcription in living cells.

WES (Whole Exome Sequencing)

Since exons comprise only about 1% of the human genome, the exome represents a much smaller and more tractable target than the complete genome.

Whole Exome sequencing has emerged as an alternative to whole genome sequencing as a means for diagnosing rare or cryptic genetic diseases.

TRANSGENIC ANIMALS

Foreign genes can be introduced into fertilized egg. Animals that develop from such fertilized egg is called transgenic animal.

The gene of interest is a cloned recombinant DNA with its own promoter and a different promoter which can be selectively regulated.

Transgenic Models of Animals

Several organisms have been studied extensively as genetic models.

Musmusculus (mouse), Drosophila melanogaster (fruit fly), Caenorhabditis elegans (nematode), Saccharomyces cerevisiae (baker's yeast), and Escherichia coli (colonic bacterium).

Uses of Transgenic Animals

- Study of DNA regulatory elements of a gene
- Study of functions of gene (role of oncogene in induction of tumorigenesis)
- Study of disorders susceptible for Gene dosage (overexpression or underexpression)
- Used as a precursor to gene therapy
- For studying the physiologic effects of insertion or deletion of a particular gene
- Providing unique genetic models for doing animal experiments in pathology and Pharmacology.

Different Strategies of Genetic Modification

- Injection of the transgene into the male pronucleus of fertilized ovum
- By homologous recombination in embryonic stem cell. This is called targeted mutagenesis
- Forward Genetics
- Animal Cloning.

Targeted Mutagenesis

Basic Principle of Targeted Mutagenesis

The normal gene in a very low percentage of ES cells may be replaced by the neo–disrupted recombinant gene (transgene) by *homologous recombination*.

Targeted Mutagenesis can be of different types:

Gene Knock out: Endogenous gene is replaced by mutated transgene by homologous recombination in embryonic stem cell.

Gene Knock in: Mutated endogenous gene is replaced by normal transgene by homologous recombination in embryonic stem cell.

Gene Knock down–si RNA or mi RNA induced gene silencing called RNA interference or RNAi

Gene Knock up: Using transcription factors, transcription of gene is increased.

Steps for Generation of a Knock out Mouse

1. Inactivating a recombinant purified gene
2. Culture embryonic stem (ES) cell from mice
3. Transfection of ES with cloned mutated non–functional gene
4. A few cultured embryonic cells contain the non–functional gene through homologous recombination.
5. Isolate ES cells with altered gene
6. Microinjection of altered ES cells into mouse-blastocyst
7. Implantation of blastocyst into foster mother
8. **Breed to many generation** till offspring with altered gene in its germ cell.

Characteristic Features of Different Strategies of Transgenic Models

1. **Transgenic: Pronuclear injection of transgene**
   - This technique is commonly used
   - Random integration of transgene
   - Genomic DNA or cDNA constructs can be injected
   - Variable copy numbers of transgene
   - Variable expression in each individual founder
   - Gain–of–function models produced due to over expression using tissue–specific promoters

- Loss–of–function models produced using antisense and dominant negative transgenes
- Inducible expression possible
- Applicable to several species.

2. Targeted mutagenesis

By homologous recombination

- Site specific integration of transgene
- Predominantly used in mice

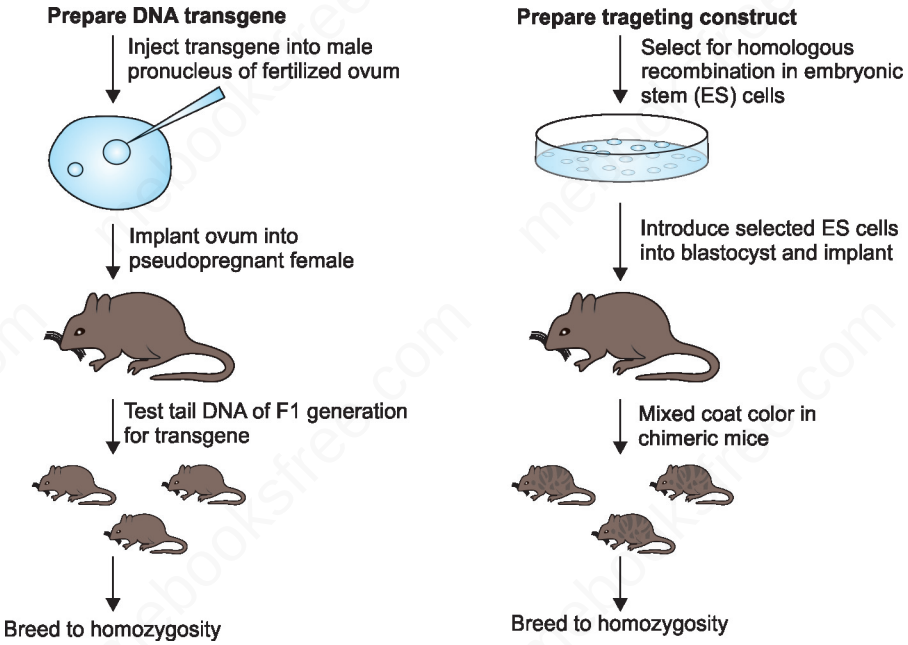


Fig. 14.9: Generation of transgenic mice by transgenesis and targeted mutagenesis

Characteristics of gene knock out

- Tissue–specific knock–out possible
- Absence of phenotype possible due to redundancy, may not be always due to knock out.

Characteristics of gene knock in

- Predominantly used in mice
- Can accurately model human disease

Latest technique of gene modulation in targeted mutagenesis are:

- miRNA/Si RNA-mediated Gene Silencing called as Gene Knock down.
- CRISPR Cas 9-mediated gene editing (loss of function of gene otherwise knock down or gain-of-function of genes)

Forward Genetics

- Mutations created randomly by ENU (N–ethyl–N–nitrourea)
- A phenotype selected
- Then its genetic characterization done
- Useful for identifying novel genes.

Animal Cloning

Nucleus of an oocyte is removed. This enucleated oocyte is fused nucleus of a somatic cell. The fused cell is implanted into the uterus of a surrogate mother. This is also called Nuclear transfer.

Characteristics of Animal Cloning

- Successful in several mammalian species including sheep, mice, cows, monkeys
- Cloning of genetically identical individuals is possible
- May affect lifespan
- Lots of Ethical concerns.

Successful Stories of Animal Cloning

- Ian Wilmut and Keith Campbell cloned sheep named Dolly in 1996
- First lamb born to Dolly is Bonnie.

Antisense Nucleic Acid in Research and Therapy

- Antisense nucleic acid can be RNA or DNA
- It can be natural or synthetic
- It is complementary to mRNA
- Si RNA can be used

Antisense nucleic acid binds with target mRNA, it is selectively destructed or inhibited

Uses

- Gene knock down to study function of a gene
- Treat viral infection like HIV.

HYBRIDOMA

- Technique to produce monoclonal antibodies in the clinical laboratory
- Introduced by Georges Kohler and Cesar Milstein in 1975
- Monoclonal antibodies are antibodies against a specific epitope of the antigen.

Steps

1. Spleen cells from immunized animal fused with mice myeloma cell to produce hybrid cell. (Polyethylene Glycol^{Q}, PEG –1500 is the fusing agent).
2. Grown in HAT (Hypoxanthine, Aminopterine and Thymidine) medium
   Unfused normal cells die as they lack multiplication potential.
   Unfused Myeloma Cells Die
   Aminopterine, a folate antagonist inhibit de novo purine synthesis and hence DNA synthesis as they lack HGPRTase enzyme.
   Fused Normal Cell and Myeloma Cell Survive
   As normal cells provide HGPRTase enzyme.
   Myeloma cell have multiplication potential.
3. Select Positive clones and expand.
4. Indefinite amount of antibodies harvested.
   Application of Monoclonal antibodies
   - Enumeration of lymphocyte subpopulation
   - Quantitative preparation of specific cells
   - Nephelometric assay of blood components
   - To prepare Monoclonal antibodies for ELISA
   - Quantitative preparation of pure antigens.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

- Commonly called as 'rif lip'

Definition

An inherited difference in the pattern of restriction map produced by the digestion of a specific restriction endonuclease is called Restriction fragment length polymorphism.

This is because of certain DNA variations present in the genome that create a new restriction site (the site where the restriction endonuclease cleave) or abolish a restriction site.

Two type of DNA Variations that result in RFLP are:

1. Single Nucleotide Polymorphism (SNP)
2. Variable Number Tandem Repeat (VNTR)

Certain Mutation can also result in RFLP if it alters the Restriction Site

Sickle cell anemia is an example of a genetic disease caused by a point mutation. The sequence altered by the mutation abolishes the recognition site of the restriction endonuclease Mst-ii.

Procedure of RFLP

- DNA is extracted from the cell
- DNA is cleaved by a specific Restriction endonuclease
- DNA fragments obtained are separated by Agarose Gel Electrophoresis
- DNA fragments are denatured
- Transferred to Nitrocellulose membrane (Southern blot)
- Treated with Radiolabelled probe
- DNA variation detected by looking for hybridization by autoradiography.

Uses of RFLP

Tracing chromosomes from parent to offspring

Prenatal diagnosis of diseases

- Direct diagnosis of sickle cell disease using RFLP
- Indirect, prenatal diagnosis of phenylketonuria

Medicolegal uses

Detect mutations, point mutation, insertion, deletion.

DNA Fingerprinting

- The use of normal genetic variation in the DNA (SNP or VNTR or RFLP) to establish a unique pattern of DNA fragments for an individual
- This is also called DNA Profiling
- Most commonly used is VNTR or repeat length polymorphism
- The process of DNA fingerprinting was invented by Alec Jeffreys in 1985.

DNA Footprinting

- DNA with protein bound is resistant to digestion by DNase enzymes
- When a sequencing reaction is performed using such DNA, a protected area, representing the 'footprint' of the bound protein, will be detected
- Because nucleases are unable to cleave the DNA directly bound by the protein.

Chromosome Walking

- Method to isolate and clone target DNA from a long segment of DNA

- In Chromosome walking, a fragment representing one end of a long piece of DNA is used to isolate another fragment, that overlaps the first but extends the first
- This process continued till the target DNA is isolated
- Cystic fibrosis (CF) gene was the first to be isolated solely by chromosome walking.

Technique of Chromosome Walking

- Gene X to be isolated from a long of DNA
- The exact location of Gene X is also not known
- A probe directed against a fragment of DNA in the 5′ end is available
- The initial probe hybridize fragment 1
- This is used to detect a probe to detect fragment 2
- This process repeated until the fragment that contain Gene X is reached.

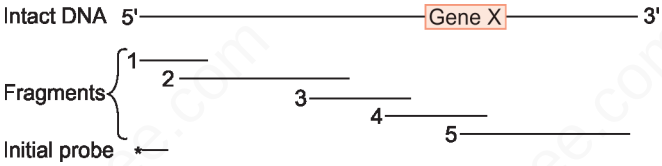


Fig. 14.10: Chromosome walking

Chromosome Jumping

- Bypass regions difficult to clone, such as those containing repetitive DNA, that cannot be easily mapped by chromosome walking.

STEM CELL BIOLOGY

Stem Cell

The most widely accepted stem cell definition is a cell with a unique capacity to produce unaltered daughter cells **(Self Renewal)** and to generate specialized cell types (potency).

Totipotent cells

- Can form an entire organism autonomously
- Only a fertilized egg (zygote) possesses this feature.

Pluripotent cells

- Can form almost all of the body's cell lineages (endoderm, mesoderm, and ectoderm), including germ cells, e.g. Embryonic Stem (ES) cells.

Multipotent cells

- Can form multiple cell lineages but cannot form all of the body's cell lineages, e.g. Hematopoetic Stem (HS) cells.

Oligopotent cells

- Can form more than one cell lineage but are more restricted than multipotent cells. Oligopotent cells are sometimes called progenitor cells or precursor cells, e.g. Neural Stem cells.

Unipotent cells or monopotent cells

- Can form a single differentiated cell lineage, e.g. spermatogonial stem (SS) cells.

Nuclear reprogramming

- The reversal of the terminally differentiated cells to totipotent or pluripotent cells.

Trans differentiation

- Lineage–committed multipotent cells, possessing the capacity to differentiate into cell types outside their lineage restrictions.

Types of Stem Cell

Name	Source	Properties
Embryonic stem cells (ES, ESC)	Blastocysts or immuno–surgically isolated inner cell mass (ICM) from blastocysts	Maintain a **stable euploid karyotype** even after extensive culture and manipulation. **Can differentiate into a variety** of cell types *in vitro*
Embryonic germ cells (EG, EGC)	Primordial germ cells (PGCs) from embryos	EG cells show essentially the same pluripotency as ES cells
Trophoblast stem cells (TS, TSC)	Trophectoderm	TS can contribute exclusively to all trophoblast subtypes when injected into blastocysts
Extra embryonic endoderm cells (XEN)	ICM from blastocysts	XEN cells can contribute only to the parietal endoderm lineage when injected into a blastocyst
Embryonal carcinoma cells (EC)	Teratocarcinoma	They can contribute to nearly all cell types when injected into blastocysts
Mesenchymal stem cells (MS, MSC)	Bone marrow, muscle, adipose tissue, peripheral blood, and umbilical cord blood	MS cells can differentiate into mesenchymal cell types, including adipocytes, osteocytes, chondrocytes, and myocytes

Contd...

Contd...

Name	Source	Properties
Multipotent adult stem cells (MAPC)	Bone marrow mononuclear cells	MAPCs can differentiate into all tissues *in vivo* when injected into a mouse blastocyst, and can differentiate into various cell lineages of mesodermal, ectodermal, and endodermal origin *in vitro*
Spermatogonial stem cells (SS, SSC)	Newborn testis	SS cells can reconstitute long–term spermatogenesis after transplantation into recipient testes and restore fertility
Germline stem cells (GS, GSC)	Neonatal testis	GS cells can differentiate into three germlayers *in vitro* and contribute to a variety of tissues, including germline, when injected into blastocysts
Multipotent adult germline stem cells (maGSC)	Adult testis	maGSC can differentiate into three germlayers *in vitro* and can contribute to a variety of tissues, including germline, when injected into blastocysts
Neural stem cells (NS, NSC)	Fetal and adult brain (subventricular zone, ventricular zone, and hippocampus)	NS cells can differentiate into neuron and glia *in vivo* and *in vitro*. Recently, the culture of pure population of symmetrically dividing adherent NS cells became possible
Unrestricted somatic stem cells (USSC)Q	Mononuclear fraction of **cord blood**	USSCs can differentiate into a variety of cell types *in vitro* and can contribute a variety of cells types in *in vivo* transplantation experiments
Epistem cells (EpiSC)	Early postimplanta-tion epiblast	EpiSCs can differentiate into three germlayers *in vitro* and form teratomas but cannot contribute to normal tissues when injected into blastocysts
Induced pluripotent stem cells (iPS, iPSC)	Variety of terminally differentiated cells and tissue stem cells.	A number of somatic cell types can be converted into iPS cells using different combinations of transcription factors and treatment with small molecule

NB: Just have an overall idea about this table given in Harrison

GENE THERAPY

Novel Area of Therapeutics

Intracellular delivery of genes to generate a therapeutic effect by correcting an existing abnormality.

Divided into

1. Somatic cell gene therapy—Gene is introduced into somatic cells.
2. Germ cell gene therapy (Transgenic animal).

Methods of Gene DeliveryQ

Chemical methods of gene delivery

DEAE–dextran

- Diethylaminoethyl–dextran (DEAE Dextran) is a poly cationic derivative of the carbohydrate polymer, dextran
- Because of its positive charge, DEAE–dextran is able to bind to the anionic phosphodiester backbone of DNA
- The resultant complex maintains an overall cationic charge and is able to bind to negatively charged cell membrane surfaces
- Subsequently, the complex is internalized, presumably by endocytosis.

Calcium PhosphateQ

Mixing DNA with calcium chloride, and then carefully adding this mixture to a phosphate buffered saline solution followed by incubation at room temperature.

This generates a DNA–containing precipitate, which is then dispersed onto cultured cells.

The precipitate is then taken into the cells via endocytosis or phagocytosis.

Advantages of the calcium phosphate method are:

- Simplicity
- Low cost
- Applicability to a wide variety of cell types
- Generate stably transfected cell lines, allowing for long–term gene expression studies.

Cationic Lipids (Lipofection)

When lipids mixed with DNA in water, the lipids formed hollow spheres, called liposomes, with DNA entrapped in the aqueous center.

When these liposomes were added to cells growing *in vitro*, some of the liposomes would fuse with cellular plasma membranes and be taken up into the cells via endocytosis.

Polymers

- More recently, a variety of organic polymers have been utilized for transfection
- One of the most popular is the polycation, poly ethylenimine (PEI)
- PEI is an organic macromolecule that possesses a high cationic charge density, sometimes known as a "proton sponge"
- It condenses DNA into positively charged particles that interact with anionic cell surfaces and enter cells via endocytosis.

Physical Methods of Gene Delivery

Microinjection

It entails the direct injection of DNA into the nuclei of target cells using fine glass needles under microscopy.

Conceptually, microinjection is the simplest gene delivery method, but one of the most difficult to apply.

Tedious and time–consuming, typically allowing only a few hundred cells to be transfected per experiment.

Electroporation[Q]

Electroporation is a method of introducing nucleic acids into cells by exposing the cells to a rapid pulse of high–voltage current, causing pores in the cell membrane to open temporarily.

This allows exogenous DNA to pass through the pores and into the cytoplasm of the cells.

Typically, the gene transfer efficiency is relatively low, and electroporation frequently results in a high incidence of cell death.

Gene Gun[Q]

Plasmid DNA is coated onto metal microparticles and then blasted into cells using either electrostatic force or gas pressure.

Some of the DNA becomes trapped by a few cells, and may then be expressed to sufficient levels.

This technique is fast, simple and safe, and it can transfer genes to a wide variety of tissues.

Also, there appears to be no limits to the size or number of genes that can be delivered.

Viral Vectors in Gene Delivery

Viral vectors	Main advantages	Main disadvantages
Retroviral	Persistent gene transfer in dividing cells	Theoretical risk of insertional mutagenesis
Lentiviral	Persistent gene transfer in transduced tissues	Might induce oncogenesis in some cases

Contd...

Contd...

Viral vectors	Main advantages	Main disadvantages
Adenoviral	Highly effective in transducing various tissues	Viral capsid elicits strong immune responses
Adeno-Associated viral	Elicits few inflammatory responses, nonpathogenic	Limited packaging capacity
Human foamy virus	Persistent gene expression in both dividing and non-dividing cells	Need a stable packaging system
HSV–1	Large packaging capacity with persistent gene transfer	Residual cytotoxicity with neuron specificity
Simian virus–40	Wide host cell range; lack of immunogenicity	Limited packaging capacity
Alpha viruses	Limited immune responses against the vector	Transduced gene expression is transient

Nonviral Vectors

Nonviral Vectors	Main advantages	Main Disadvantages
Transposon/ Transposase system	1. Undetermined, probably large packaging capacity. 2. Transfects many cell types with long–term gene expression	Early stage in development
Liposome	1. Undetermined, probably large packaging capacity. 2. Transfects many cell types. Large holding capacity to enable a high number of base pairs	Expensive to produce
Naked DNA	1. Undetermined, probably large packaging capacity. 2. Efficient in gene transfer. 3. Limited immunogenicity	Transient and low–level expression
Site Specific Integrase	1. Undetermined, probably large packaging capacity. 2. Specific integration site	Early stage in development

Strategies of Gene Transfer

1. ***In vivo***
   Gene is transferred directly into the patient.
2. **Ex vivo**
   Removal of target cell.
   Gene transfer into target cells.
   Return of target cell into the patient after gene transfer.

Application of Gene Therapy in Medicine

Gene therapy clinical trials addressed in:

1. Cancer (67%)—Most common
2. Vascular Disease (8.9%)
3. Monogenic Disorders (8.6%)

Gene Therapy in Cancers

Local/Regional Approach

Suicide gene/prodrug

- Intratumoral injection of an adenoviral vector expressing the thymidine kinase (TK) gene
- Cells that take up and express the TK gene are susceptible to Gancyclovir.

Used for the treatment of:

- Glioblastoma multiforme
- Locally recurrent tumors of prostate, breast, and colon.

Suppressor oncogene

Adenoviral–mediated expression of the tumor suppressor gene for p53

- Squamous cell carcinoma of the head and neck
- Esophageal cancer
- Non–small cell lung cancer

Oncolytic viruses

These selectively replicate in tumor cells and not in normal cells.

- Squamous cell carcinoma of the head and neck.

Systemic Gene Therapy

Immunomodulation

Immune–enhancing genes encoding cytokines, chemokines, or co–stimulatory molecules.

Anti–angiogenesis

- Constitutive expression of angiogenesis inhibitors such as angiostatin and endostatin
- Use of siRNA to reduce levels of VEGF or VEGF receptor.

Gene Therapy for Vascular Disease

Transgene for VEGF, fibroblast growth factor (FGF) and hypoxia–inducible factor which increase blood flow.

- Skeletal muscle (critical limb ischemia)
- Cardiac muscle (angina/myocardial ischemia).

Gene Therapy for Genetic Disorders

- Gene transfer strategies for genetic disease generally involve gene addition therapy
- This approach most commonly involves transfer of the missing gene to a physiologically relevant target cell
- First genetic disorder addressed by gene therapy is Severe Combined Immunodeficiency
- Father of Gene therapy is French Anderson.

Gene Therapy for Other Disease

- Parkinson's disease: AAV vectors expressing enzymes required for enhanced production of dopamine, or of the inhibitory neurotransmitter Gamma aminobutyric acid
- In Alzheimer's disease, fibroblasts are transduced with a retroviral vector expressing nerve growth factor.

HUMAN GENOME PROJECT

In 1990, the United States launched a multibillion dollar effort, the **Human Genome Project**, for the express purpose of developing the automated **high–throughput** techniques, instrumentation, and data mining software necessary to determine the entire DNA sequence of the Homo sapiens genome.

Bioinformatics

The discipline concerned with the collection, storage, and analysis of biologic data, mainly DNA and protein sequences.

"Omics" of Molecular Biology

Genome: The complete set of genes of an organism

Genomics: In depth study of the structures and functions of genomes.

Transcriptome: The complete set of RNA transcripts produced by the genome during a fixed period of time.

Transcriptomics: The comprehensive study of transcriptome.

Proteome: The complete complement of proteins of an organism.

Proteomics: The systematic study of structures and functions of proteomes and their variation in health and disease.

Glycome and Glycomics: The glycome is the total complement of simple and complex carbohydrates in an organism. Glycomics is the systematic study of the structures and functions of glycomes such as the human glycome.

Lipidome and Lipdomics: The lipidome is the complete complement of lipids found in an organism. Lipidomics is the in–depth study of the structures and functions of all members of the lipidome and of their interactions, in both health and disease.

Metabolome and Metabolomics: The metabolome is the complete complement of metabolites (small molecules involved in metabolism) present in an organism. Metabolomics is the in–depth study of their structures, functions, and changes in various metabolic states.

Contd...

Contd...

Nutrigenomics: The systematic study of the effects of nutrients on genetic expression and of the effects of genetic variations on the metabolism of nutrients.

Pharmacogenomics: The use of genomic information and technologies to optimize the discovery and development of new drugs and drug targets

Exome: The nucleotide sequence of the entire complement of mRNA exons expressed in a particular cell, tissue, organ or organism.

TECHNIQUES TO STUDY PROTEOMICS

First generation Proteomics

- To purify proteins sample–SDS PAGE or 2D Electrophoresis
- To determine the amino acid sequence–End group analysis like Sanger's reagent and Edman's reagent.

Second generation Proteomics

To purify protein in the sample Nanoscale Chromatographic techniques

To determine amino acid sequence:

- Mass Spectrometry
- Mud PIT (Multidimensional Protein Identification Technology)

MudPIT (Multidimensional Protein Identification Technology)

Successive rounds of chromatography to resolve the peptides produced from complex biologic sample into simpler fractions, that can be analyzed separately by Mass Spectrometry.

BIOINFORMATIC AND GENOMIC RESOURCES

The large collection of databases that helps in contemporary molecular, biochemical, epidemiological, and clinical research.

UniProt KB

The UniProt Knowledgebase, UniProtKB, is jointly sponsored by the Swiss Institute of Bioinformatics and the European Bioinformatics Institute.

UniProtKB's stated objective is "to provide the scientific community with a comprehensive, high–quality and freely accessible resource of protein sequence and structural information".

It is organized into two sections:

- **Swiss–Prot** contains entries whose assigned functions, domain structure, post–translational modifications, etc. have been verified by **manual curation**
- **TrEMBL**, on the other hand, contains empirically determined and genome derived protein sequences whose potential functions have been assigned, or annotated, automatically—solely on the basis of computer algorithms

Thus, while TrEMBL currently includes more than 80 million entries, Swiss–Prot contains slightly more than 500,000.

Gen Bank

- The genetic sequence database
- The goal of GenBank, of the National Institutes of Health (NIH), is to collect and store all known biological nucleotide sequences and their translations in a searchable form.

PDB

- The Protein Data Base (PDB) is a repository of the three dimensional structures of proteins polynucleotides, and other biological macromolecules.
- The PDB presently contains over 95,000 three–dimensional structures for proteins.

Tagged SNPs

- When sets of SNPs localized to the same chromosome are inherited together in blocks, the pattern of SNPs in each block is termed a **haplotype**
- **Tag SNPs, is** a subset of the SNPs in a given block sufficient to provide a unique marker for a given haplotype.
- Selected regions are then subject to more detailed study to identify the specific genetic variations that contri-bute to a specific disease or physiologic response.

HapMap

- **Haplotype Map (HapMap) Project**, a comprehensive effort to identify SNPs associated with common human diseases and differential responses to pharmaceuticals
- The long–term goal of the project is to provide earlier and more accurate diagnosis of potential genetic risk factors that lead to improved prevention and more effective patient management.

ENCODE

- National Human Genome Research Institute (NHGRI) initiated the **ENCODE (Encyclopedia of DNA Elements) Project**
- ENCODE is a collaborative effort that combines laboratory and computational approaches to identify every functional element in the human genome
- These include mapping sites of DNA methylation, assessing local histone methylation etc.

Entrez Gene

***Entrez* Gene,** a database maintained by the National Center for Biotechnology Information (NCBI).

- This provides a variety of information about individual human genes
- The information includes the sequence of the genome in and around the gene, exon–intron boundaries, the sequence of the mRNA (s) produced from the gene, and any known phenotypes associated with a given mutation of the gene in question.

dbGAP

- dbGAP, the Database of Genotype and Phenotype, is an NCBI database that complements Entrez Gene.
- dbGAP compiles the results of research into the links between specific genotypes and phenotypes.

LINKAGE AND ASSOCIATION STUDIES

Principle of Linkage analysis and Association studies

Based on the concept of linkage

- When two genes are close together on a chromosome, they are transmitted together unless a recombination event separates them
- Genes which are close together are less likely to be separated by a recombination event.

Linkage and Association Studies

There are two primary strategies for mapping genes that cause or increase susceptibility to human disease:

1. Classic linkage analysis can be performed based on a known genetic model or, when the model is unknown, by studying pairs of affected relatives
2. Disease genes can be mapped using allelic association studies (Genome Wide Association Studies, GWAS).

Linkage Analysis

- The genetic marker loci very close to the disease allele, that are transmitted through pedigrees called linkage disequilibrium
- Assess the genetic marker loci in **family members** having the disease or trait of interest
- With time it becomes possible to define a panel of marker loci, all of which co–segregate with the putative diseases
- Hence, Linkage analysis facilitates localization and cloning of the disease allele
- The genetic markers used are, SNPs and repeat–length polymorphisms (minisatellite and microsatellite repeats).

GWAS

- In GWAS large cohorts of patients with and without a disease (rather than families) are examined across the entire genome for genetic variants or polymorphisms that are over–represented in patients with the disease
- This identifies regions of the genome that contain a variant gene or genes that confer disease susceptibility
- The causal variant within the region is then provisionally identified using a "candidate gene" approach
- By this approach, in the provisionally identified region, genes are selected based on how tightly they are associated with the disease and whether their biologic function seems likely to be involved in the disease under study
- Thus, localize the causal gene or in some instances, functional polymorphism associated with it.

Comparison of Linkage and Association Studies

Linkage Study	Association Study
Study conducted in the same family or same sibships	Compare a population of affected individuals with a control population
Analysis of monogenic traits	Suitable for identification of susceptibility genes in polygenic and multifactorial disorders
Difficult to obtain sufficient statistical power for complex traits	More statistical power for complex **multigenic disorder**

REVIEW QUESTIONS

Recombinant DNA Technology

1. cre-cis regulatory elements bind to what site? (AIIMS May 2015)

a. RE site
b. FTR site
c. Lox b4 site
d. INT site

Ans. c. Lox b4 site *(Ref: Harper 30/e page 454)*

Recombinases	Recognition site
CRE Recombinase	Bacterial Lox P site
λ phage encoded INT protein	Bacteriphage λ tt site
Yeast Flp Recombinases	Yeast FRT site

2. cDNA used in gene amplification in bacteria of genomic DNA because: (PGI May 2014)

a. Easy to replicate
b. Human genome has many introns that cannot be removed by bacteria
c. Promoter are not found
d. Complete genome cannot be replicated

Ans. a, b, d. Easy to replicate, Human genome has many introns that cannot be removed by bacteria, Complete genome cannot be replicated *(Harper 30/e page 455)*

cDNA

DNA complementary to mRNA is called cDNA or Copy DNA or Complementary DNA.

Procedure to prepare cDNA

- Isolate the mRNa
- By the action of Reverse Transcriptase RNA-DNA hybrid is synthesized
- By RNAse H RNA is digested
- By DNA Polymerase double stranded DNA is produced.

Advantages of cDNA over genomic DNA

- Contains only coding sequences
- Represent the mRNA in a tissue
- Hence used to study gene expression.

3. Restriction Endonuclease is used in (JIPMER 2013)

a. RFLP
b. PCR
c. FISH
d. SDS-PAGE

Ans. a. RFLP

In RFLP, DNA is cut using a Restriction endonuclease. They cut DNA at specific pallindromic sites. So it produces a characteristic restriction map.

4. Function of endonucleases (TN 97)

a. Cut DNA at specific DNA sequences
b. To point out the coding regions
c. Enhancers
d. To find out antibiotic resistance

Ans. a. Cut DNA at specific DNA sequence *(Ref: Harper 30/e page 453)*

- Restriction endonuclease cut DNA at specific palindromic sites
- These enzymes are isolated from bacteria
- They restrict the entry of phages into the bacteria.

5. Enzymes used in DNA research programme are, except: (PGI June 97)

a. Polymerase
b. Exonuclease
c. Nuclease
d. Alkaline phosphatase
e. None

Ans. e. None *(Ref: Harper 30/e page 453)*

Enzyme	Reaction	Uses
Alkaline phosphatase	Dephosphorylates 5' ends of RNA and DNA	Removal of 5'-PO_4 groups prior to kinase labeling; also used to prevent self-ligation
DNA ligase	Catalyzes bonds between DNA molecules	Joining of DNA molecules
DNA polymerase I	Synthesizes double-stranded DNA from single-stranded DNA	Synthesis of double-stranded cDNA; Nick translation; Generation of blunt ends from sticky ends
Thermostable DNA polymerases (Taq Polymerase)	Synthesize DNA at elevated temperatures (60–80° C)	Polymerase chain reaction (DNA synthesis)
DNase I	Under appropriate conditions, produces single-stranded nicks in DNA	Nick translation Mapping of hypersensitive sites Mapping protein-DNA interactions
Exonuclease III	Removes nucleotides from 3' ends of DNA	DNA sequencing Mapping of DNA-protein interactions

Contd...

Contd...

Enzyme	Reaction	Uses
λ Exonuclease	Removes nucleotides from 5' ends of DNA	DNA sequencing
Polynucleotide kinase	Transfers terminal phosphate (ɣ position) from ATP to 5'-OH groups of DNA or RNA	^{32}P end-labeling of DNA or RNA
Reverse transcriptase	Synthesizes DNA from RNA template	Synthesis of cDNA from mRNA; RNA (5' end) mapping studies
S1 nuclease	Degrades single-stranded DNA	Removal of 'hairpin' in synthesis of cDNA; RNA mapping studies (both 5' and 3' ends)
Terminal transferase	Adds nucleotides to the 3' ends of DNA	Homopolymer tailing
CRISPR-Cas9	RNA targeted DNA directed Nuclease	Genome deiting and modulation of gene expression

6. In DNA transfer, the vectors used from smallest to largest is: (PGI Dec 07)

a. Cosmids, Plasmids, Bacteriophage
b. Plasmids, Bacteriophage, Cosmids
c. Bacteriophage, Cosmids, Plasmids
d. Cosmids, Bacteriophage, Plasmids
e. Plasmids, Cosmids, Bacteriophage

Ans. b. Plasmids, Bacteriophage, Cosmids

(Ref: Harper 30/e page 455)

The DNA insert size in ascending order is Plasmid < Phage < Cosmids < BAC/PAC < YAC Cloning capacity of common cloning vectors

Vector	DNA insert size (Kbp)
Plasmid	0.01–10
Lambda Phage	10–20
Cosmid	35–50
BAC, PAC	50–250
YAC	500–3000

7. In gene cloning, largest fragment can be incorporated in: (AIIMS Dec 95)

a. Plasmid
b. Bacteriophage
c. Cosmid
d. Retrovirus

Ans. c. Cosmid *(Ref: Harper 30/e page 455)*

8. Function of restriction II enzyme (AI 2012)

a. Prevents protein folding
b. Removing formed DNA
c. Cleaves DNA at palindromic recognition site
d. Negative supercoiling

Ans. c. Cleaves DNA at palindromic recognition site.

(Ref: Harper 30/e page 452)

9. After digestion by restriction endonucleases DNA strands can be joined again by: (AIIMS May 2011) (Nov 2010)

a. DNA polymerase
b. DNA ligase
c. DNA topoisomerase
d. DNA gyrase

Ans. b. DNA ligase *(Ref: Harper 30/e page 453)*

Enzymes Involved in the DNA Replication

1. **Topoisomerases**
   - Relieve torsional strain that results from helicase-induced unwinding of DNA
   - Nicking Resealing Enzyme
2. **Helicase:** ATP driven processive unwinding of DNA
3. **Single Strand Binding Protein (SSB)** Prevent premature reannealing of dsDNA
4. **DNA Primase**
   - Initiates synthesis of RNA primers

   Special class of DNA dependent RNA Polymerase.
5. **DNA Polymerase:** Catalyse the chemical reaction of DNA Polymerization. Synthesize DNA only in 5′ to 3′ direction.
6. **DNA Ligase:** Seals the single strand nick between the nascent chain and Okazaki fragments on lagging strand.

10. Starting material for production of insulin from bacteria is: (AIIMS May 2011)

a. Genomic DNA of lymphocytes
b. mRNA of lymphocytes
c. Genomic DNA of beta cell of pancreas
d. mRNA of beta cells of pancreas

Ans. d. mRNA of beta cells of pancreas

(Ref: Vasudevan and Sreekumari 7/e page 626)

11. True statement about Restriction Endonuclease: (PGI May 2012)

a. Palindromic Sequence Observed
b. Protects bacteria from infection by virus
c. Present only in Eukaryotes
d. Restrict replication of DNA

Ans. a, b. Palindromic Sequence Observed (b) Protects bacteria from infection by virus.

(Ref: Harper 30/e page 452)

Restriction Enzymes

- Recognizes and cleaves a specific palindromic double-stranded DNA sequence that is typically 4–7 bp long.
- These DNA cuts result in **blunt ends** (*e.g. HpaI*) overlapping **(sticky or cohesive) ends** (*e.g, BamHI*)
- Are a key tool in recombinant DNA research.
- These enzymes were called **restriction enzymes** because their presence in a given bacterium restricted the growth of certain bacterial viruses called bacteriophages.
- Each enzyme recognizes and cleaves a specific double-stranded DNA sequence that is typically 4–7 bp long.
- Restriction endonucleases are present only in cells that also have a companion enzyme, **site-specific DNA methylases**, that site-specifically methylates the host DNA, rendering it an unsuitable substrate for digestion by that particular restriction enzyme. Thus, prevent the digestion of host DNA.

Restriction Enzymes are Named after the Bacterium from which They are Isolated

For example, EcoRI is from *Escherichia coli*, and BamHI is from Bacillus amyloliquefaciens.

The first three letters in the restriction enzyme name consist of the first letter of the genus (*E*) the first two letters of the species (*co*). These may be followed by a strain designation (*R*)

A roman numeral (*I*) to indicate the order of discovery (e.g. EcoRI and EcoRII).

12. True about Gene Library: (PGI Nov 2009)

a. Library of Gene books
b. Plasmid with copies of different genes
c. Computer database with all gene knowledge
d. Collection of gene copies of one organism as completely as possible in bits and pieces.
e. DNA fragments.

Ans. d, e. Collection of gene copies of one organism as completely as possible in bits and pieces, DNA fragments.

(Ref: Harper 30/e page 455)

Gene Library

A collection of recombinant DNA clones generated from a specific source.

Two types of Gene Library:

1. Genomic DNA Library:
   Prepared from total genomic DNA of an organism.
   By digestion of Genomic DNA by Restriction Endonuclease.
   Then recombinant clones of such digested DNA is produced by recombinant DNA technology.
2. cDNA library
   cDNA is prepared from mRNA by the action of Reverse Transcriptase
   The recombinant clones for cDNA are produced by Recombinant DNA Technology.

Advantages of cDNA over Genomic DNA

- Contains only coding sequences.
- Represent the mRNA in a tissue.
- Hence, used to study gene expression.

13. Restriction enzymes: (PGI Nov 2009)

a. Prevent elongation of DNA
b. Break DNA to create sticky end
c. Cuts at palindromic sites
d. Restriction sites are not specific
e. Breaks at sugar-phosphate bond

Ans. a, b, c, e. Prevent elongation of DNA, Break DNA to create sticky end, Cuts at palindromic sites, breaks at sugar-phosphate bond. *(Ref: Harper 30/e page 455)*

14. Correct statements regarding restriction endonuclease is/are: (PGI Dec 03)

a. Restriction endonuclease recognizes specific sites of DNA sequence
b. Restriction endonuclease recognizes short sequence of DNA
c. It acts at 5′ – 3′ direction
d. It acts at 3′ – 5′ direction

Ans. a. Restriction endonuclease recognizes specific sites of DNA sequence. *(Ref: Harper 30/e page 457)*

15. True about the function of Restriction endonuclease: (PGI Dec 06)

a. Cut both the strands of ds DNA
b. The cut ends produced are sticky
c. The cut ends produced are blunt
d. Cuts single strand of DNA

Ans. a, b, c. Cut both the strands ..., The cut ends produced are sticky. The cut ends produced are blunt.

(Ref: Harper 30/e page 457)

Restriction endonuclease cut both strands of DNA. They produce sticky ends or blunt ends depending on which restriction endonuclease act on the DNA.

PCR

16. Real-time PCR is used for: (AIIMS May 2013)

a. Multiplication of RNA
b. Multiplication of specific segment of DNA
c. Multiplication of Protein
d. To know how much amplification has occurred

Ans. d. To know how much amplification has occurred

(Ref: Tietz Textbook of Clinical Chemistry 4/e page 1446)

- Real-Time (Homogeneous, Kinetic) Polymerase Chain Reaction
- Real-time PCR describes methods by which the target amplification and detection steps occur simultaneously in the same tube. Thus it is a method of quantitative PCR or q PCR.
- Quantitaion is done in the exponential phase of amplification.

17. Quantitative DNA analysis/estimation is done by: (AIIMS May 2012)

a. pH meter
b. Sphymometer
c. Spirometer
d. Spectrometer

Ans. d. Spectrometer

- Absorbance of UV light at 260 nm can be used to estimate DNA
- Can be done using spectrophotometer or simply spectrometer.
- Other options are self-explanatory.

18. All are added to PCR, except: (AIIMS Nov 2012)

a. Deoxynucleotide
b. Dideoxynucleotide
c. Thermostat DNAP
d. Template DNA

Ans. b. Dideoxynucleotide

(Ref: Tietz Textbook of Clinical Chemistry 4/e page 1446)

Pre-requisites of PCR

- Sample DNA to be amplified
- Deoxynucleotides
- Thermostable Polymerase-Taq Polymerase obtained from Thermus Aquaticus found in hot springs.
- Primer.
- $MgCl_2$, KCl

19. For PCR which of the following is not required: (AIIMS May 2007)

a. Taq polymerase
b. d-NTP
c. Primer
d. Radiolabelled DNA probe

Ans. d. Radiolabelled DNA Probe

(Ref: Tietz Textbook of Clinical Chemistry 4/e page 1446)

20. SYBR Green Dye is used for: (AIIMS May 2008)

a. HPLC
b. Immunofluorescence
c. PCR
d. ELISA

Ans. c. PCR *(Ref: Tietz Textbook of Clinical Chemistry 4/e page 1446)*

Real-time PCR

- This is a type of Quantitative PCR.
- **Methods used to quantitate PCR Products in Real time PCR**
- Intercalating Dyes—Ethidium Bromide, SYBR Green.
- Sequence Specific Probes—TaqMan, Molecular beacon
- Fret Probes—Fluorescence Resonance Energy Transfer Probes.

21. True about DNA polymerase used in PCR (PGI May 2013)

a. Obtained from virus
b. Obtained from bacteria
c. Used for joining two strands
d. It is heat stable
e. Add nucleotide

Ans. b, d, e. Obtained from bacteria. It is heat stable, Add nucleotide *(Ref: Tietz Textbook of Clinical Chemistry 4/e page 1446)*

DNA Polymerase used in PCR is Taq Polymerase. This is thermostable polymerase obtained from bacteria Thermophilus Aquaticus. Taq Polymerase add nucleotide during extention of PCR Cycle.

22. Enzyme(s) used in polymerase chain reaction is/are: (PGI May 2011)

a. Restriction endonuclease
b. DNA polymerase
c. Alkaline phosphate

d. RNA polymerase
e. Reverse transcriptase

Ans. b. DNA Polymerase *(Ref: Tietz Textbook of Clinical Chemistry 4/e page 1446)*

Pre-requisites of PCR:

- Sample DNA to be amplified
- Deoxy nucleotides.
- Thermostable Polymerase—Taq Polymerase obtained from ThermusAquaticus found in hot springs.
- Primer
- Mg Cl_2

23. True about PCR all except: (PGI June 2009)

a. Carried out by thermostable DNA-polymerase
b. Exponential amplification
c. Additive amplification
d. Specific amplification
e. Single-stranded DNA required

Ans. a. Additive amplification.

Polymerase Chain Reaction

- Revolutionary technique invented by Karry B Mullis in 1989.
- He got nobel prize for this in 1993.
- The polymerase chain reaction (PCR) is a test tube method for amplifying a selected DNA sequence.

Exponential amplification of the sample

- The number of samples after 'n' number of cycles is 2^n.
- One cycle of PCR require 20–30 seconds.
- 20 cycles result in million-fold amplification of the target DNA.
- Product obtained by amplification is called Amplicon.
- The instrument that takes samples through the multiple steps of changing temperature in PCR Cycle is called Thermocycler.

24. PCR is used in: (Ker 2006)

a. Medicolegal cases
b. Amplification of gene
c. Identification of organism
d. All of the above

Ans. d. All of the above *(Ref: Tietz Textbook of Clinical Chemistry 4/e page 1446)*

25. Which of the following is used in PCR? (AIIMS Nov 07)

a. Ca ++
b. Mg ++
c. Li +
d. Na +

Ans. b. Mg++ *(Ref: Tietz Textbook of Clinical Chemistry 4/e page 1446)*

26. In PCR Acquaticus thermophilus is preferred over *E coli*, because: (PGI Dec 07)

a. Thermostable at temperature at which DNA liquefies
b. Proofreading done
c. Done in more precisely
d. Does not require primer
e. Better DNA replication

Ans. a. Thermostable at temperature at which DNA liquefies *(Ref: Tietz Textbook of Clinical Chemistry 4/e page 1446)*

27. Which of the following is not true about PCR: (PGI 2003)

a. Thermostable enzyme is used
b. Annealing is done after DNA denaturation
c. Specific primers are required
d. Required at least 1st week time for synthesis
e. DNA polymerase has to be added to each cycle

Ans. d, e. Required at least 1st week time for synthesis, DNA polymerase has to be added to each cycle. *(Ref: Tietz Textbook of Clinical Chemistry 4/e page 1446)*

28. In PCR true is/are: (PGI June 08)

a. Thermostable enzyme is needed
b. 2^n copies formed after 'n' numbers of Cycle
c. Non specific
d. Thermolabile enzyme
e. Primer is needed

Ans. a, b, e. Thermostable enzyme is needed, 2^n copies formed after 'n' numbers of Cycle, Primer is needed

Blot Techniques

29. Western blot detects (AIIMS Nov 2009)

a. DNA
b. RNA
c. Protein
d. mRNA

Ans. c. Protein *(Ref: Harper 30/e page 457)*

Blot Techniques

Southern Blot

- Devised by Edward Southern in 1975

- Technique to detect specific DNA Segment
- Principle: Based on specific base pairing rule of complementary nucleic acid strands
- It is a DNA-DNA Hybridization.

Northern Blot
- Technique used to detect specific RNA
- Principle: RNA-DNA hybridization technique
- Radioactive labeled cDNA Probes used.

Western Blot (Immunoblot) analysis for Proteins
- Technique to detect specific protein in a sample
- Antigen antibody interaction
- Radioactive labeled antibody used.

South Western Blotting
- To examine Protein–DNA Interaction
- Dot blot Technique The step, blotting to nitrocellulose membrane is avoided
- The sample is directly applied to slots on a specific blotting apparatus containing nylon membrane. This is also called *slot blot*.

30. Confirmatory test for proteins are: **(PGI May 2014)**

a. Western Blot
b. ELISA
c. Chip assay
d. Dot blot

Ans. a, b, c, d. Western blot, ELISA, Chip assay and dot blot is based on Antigen antibody interaction. Hence, they are confirmatory test for proteins. Chip is the other name for Microarray. Just like DNA Chip, where DNA – DNA Hybridization is done, there Protein Microarray or Protein Chip where Antigen antibody interaction is done.

Dot blot technique: In blotting technique, the blotting to nitrocellulose membrane is avoided, instead they are arranged in different slots. This is called dot blot technique. This is applied to detect protein.

31. Which is the test used to identify mRNA **(JIPMER Nov 2015)**

a. Southern Blot
b. Northern Blot
c. Western Blot
d. South Western Blot

Ans. b. Northern blot
- Southern blot detect DNA by DNA-DNA hybridization
- Northern blot detect RNA by RNA-cDNA hybridization
- Western blot detect Protein by Antigen antibody Interaction.

Cytogenetic Techniques

32. Which method is used to locate a known gene locus? **(AIIMS May 2013)**

a. FISH
b. CGH
c. Chromosome Painting
d. RT-PCR

Ans. a. FISH *(Ref: Robbins 9/e page 177)*

Robbins 9th edition gives the following description:
- FISH uses DNA probes that recognize sequences specific to particular chromosomal regions.
- FISH requires prior knowledge of the one or few specific chromosomal regions suspected of being altered.
- Genomic abnormalities can also be detected without prior knowledge of what these aberrations may be, using a global strategy such as array CGH.
- From the above description it is concluded that
- To locate a known gene locus FISH can be used.
- To locate an unknown gene locus, a global strategy like CGH is used.

33. Light microscopy resolution to visualise chromosomes: **(AIIMS May 2013)**

a. 500 kb
b. 5 mb
c. 50 mb
d. 5 kb

Ans. b. 5 mb

(Ref: Emery's Elements of Medical Genetics 13th edition page 33)

G Banding generally provides high quality Chromosome analysis with approximately 400-500 bands per haploid set. Each of these band corresponds on an average to approximately 6000-8000 kilobases (i.e. 6-8 mb)

Method	Requires growing cells	Detects deletion and duplication	Detects balanced structural rearrangements	Detects Uniparental Disomy	Lower limits of detection
G Banding	Yes	Yes	Yes	No	5–10 mb
Metaphase FISH	Yes	Yes	Yes	No	40–250 thousand mb
Interphase FISH	No	Yes	Some	No	40–250 thousand mb
CGH array	No	Yes	No	No	Single Exon or Single gene
SNP array	No	Yes	No	Some	Single Exon or Single gene

34. Test to differentiate in the chromosome of normal and cancer cell: (AIIMS Nov 2012)

a. PCR
b. Comparative Genomic Hybridization
c. Western Blotting
d. Southern Blotting

Ans. b. Comparative Genomic Hybridization

(Ref: Robbins and Cotran Pathologic basis of disease 9/e Page 178, 179)

Array-Based Comparative Genomic Hybridization (Array CGH)

- This is also a hybridization technique done on a Microarray or DNA Chip, hence in the name array-based.
- Here two Genomes are compared, hence the name Comparative Genomic Hybridization.

Uses of array CGH

- Detect Gene Amplification (e.g. Microduplication)
- Detect Gene Deletion. (eg. Subtelomeric deletion, Microdeletion)
- Detect Copy number variations
- Detect Aneuploidy
- Hence, used diseases of unknown etiology like Cancer, Autism, Mental Retardation, Child with dysmorphic features etc.

35. Karyotyping under light microscopy is done by: (AIIMS Nov 2009)

a. R-banding
b. Q-banding
c. G-banding
d. C-banding

Ans. c. G-Banding

(Ref: Emery's Elements of Medical Genetics 13/e page 30, 31)

R-Banding, G-Banding and C-Banding are done under light microscopy.

As G-Banding is the most common method it is the correct answer.

36. Rapid method of chromosome identification in intersex is: (AIIMS May 2008)

a. FISH
b. PCR
c. SSCP
d. Karyotyping

Ans. a. FISH *(Robbins 9/e page 177, 178)*

Certain probes used in FISH hybridize to repetitive sequences located to the pericentromeric regions. These probes are useful for the rapid identification of certain trisomies in interphase cells of blood smears, or even in the rapid analysis of prenatal samples from cells obtained through amniocentesis. Such probes are available for chromosomes 13, 18, and 21 and for the sex pair X and Y.

Interphase FISH[Q]

- Allows rapid diagnosis within 24–48 hours.
- Especially useful for amniocytes.

37. Which of following techniques is used for detection of variation in DNA sequence and Gene expression: (AI 2010)

a. Northern Blot
b. Southern Blot
c. Western Blot
d. Microarray

Ans. d. Microarray *(Robbins 9/e page 177, 178)*

- DNA microarray can be used to detect DNA sequence variations
- RNA microarray can detect Gene expression.

38. Which of the following tests is not used for detection of specific aneuploidy (AI 2010)

a. FISH
b. RT-PCR
c. QF-PCR
d. Microarray

Ans. b. RT-PCR

(Henrys Clinical Diagnosis and Management by Laboratory Methods 22/e 1294.1297) Use of a

DNA method, QF-PCR, in the prenatal diagnosis of fetal aneuploidies. J Obstet Gynaecol Can. 2011 Sep; 33(9):955-60.

- Quantitative Fluorescent PCR is a real-time PCR in which fluorescent dyes are used for quantitation
- RT–PCR is used to amplify a RNA target. Not a method to detect aneuploidy.

Methods of aneuploidy detection

- Conventional Karyotyping
- FISH
- Microarray based CGH
- Multiple ligation dependent Probe Amplification (MLPA)
- Real-time PCR
- Quantitative Fluorescent PCR (QF-PCR)
- Multiplex amplifiable probe hybridization (MAPH).

39. For isolating a gene of long DNA molecules (50-100 KB) following is used: (AIIMS Dec 98)

a. Chromosome walking
b. Nitch
c. RFLP
d. SSLP

Ans. a. Chromosome walking

(Ref: Harper 30/e page 463)

Chromosome Walking

- Method to isolate and clone target DNA from a long segment of DNA
- In Chromosome walking, a fragment representing one end of a long piece of DNA is used to isolate another fragment, that overlaps the first but extends the first. This process continued till the target DNA is isolated.
- Cystic fibrosis (CF) gene was the first to be isolated solely by chromosome walking.

Mutation Detection Techniques

40. Techniques used to detect Gene Mutation is/are: (PGI May 2012)

a. RTPCR
b. Denaturing Gradient Gel Electrophoresis
c. DNA Sequencing
d. Restriction Fragment Length Polymorphism
e. Single Strand Conformational Polymorphism

Ans. a, b, c, d, e.

Test to Detect Mutations

1. Ames Test, Test to detect mutagenicity
   Special strains of *Salmonella typhimurium* have mutated histidine gene.
   Hence, they will grow only in medium containing histidine gene.
   This is called reverse mutation.
   The number of colonies is proportional to the quantity of mutagens.
2. Site Directed Mutagenesis
   Michael Smith in 1993
   An oligodeoxyribonucleotide whose sequence is complementary to a part of known gene is synthesized.
   A specific deletion/insertion is produced in the oligodeoxynucleotide.
   It is then extended by DNAP.
   This altered gene is amplified and expressed by insertion into a cloning vector.
   This allows the study of a particular mutation.

Other Mutation Detection Technique

Method	Type of Mutation Detected
Cytogenetic analysis	Numerical or structural abnormalities in chromosomes
Fluorescent in situ hybridization (FISH)	Numerical or structural abnormalities in chromosomes
Southern blot	Large deletion, insertion, rearrangement, expansions of triplet repeat, amplification
Polymerase chain reaction (PCR)	Expansion of triplet repeats, variable number of tandem repeats (VNTR), gene rearrangements, translocations; prepare DNA for other mutation methods
Reverse transcriptase PCR (RT-PCR)	Analyze expressed mRNA (cDNA) sequence; detect loss of expression
DNA sequencing	Point mutations, small deletions and insertions
Restriction fragment polymorphism (RFLP)	Point mutations, small deletions and insertions
Single-strand conformational polymorphism (SSCP)	Point mutations, small deletions and insertions
Denaturing gradient gel electrophoresis (DGGE)	Point mutations, small deletions and insertions
RNAse cleavage	Point mutations, small deletions and insertions
Oligonucleotide specific hybridization (OSH)	Point mutations, small deletions and insertions

Contd...

Contd...

Method	Type of Mutation Detected
Microarrays	Point mutations, small deletions and insertions Genotyping of SNPs
Protein truncation test (PTT)	Mutations leading to premature truncations
Pyrosequencing	Sequencing of whole genomes of microorganisms, resequencing of amplicons
Multiplex ligation-dependent probe amplification (MLPA)	Copy number variations

41. Correct statement about Restriction fragment gene: (PGI May 2011)

a. Detected by Southern blot
b. Detected by Northern blot
c. Used for identification of gene for genomic mapping
d. RFLP is DNA variation sequence

Ans. a, c, d. Detected by Southern blot, Used for identification of gene for genomic mapping, RFLP is DNA variation sequence *(Ref: LippincottIllustrated Biochemistry 6/e page 475)*

The procedure of RFLP

- DNA is extracted from the cell
- DNA is cleaved by a specific restriction endonuclease
- DNA fragments obtained are separated by Agarose Gel Electrophoresis
- DNA fragments are denatured
- Transferred to Nitrocellulose membrane (Southern blot)
- Treated with Radiolabelled probe
- DNA variation detected by looking for hybridization by autoradiography.

42. DNA fingerprinting was found by: (PGI June 06)

a. Watson
b. Galton
c. Jeffrey
d. Sanger
e. Wilkins

Ans. c. Jeffrey

43. The following methods can be used to detect the point mutation in the beta globulin gene that causes sickle cell anemia, except: (AI 06)

a. Polymerase chain reaction with allele—specific oligonucleotide hybridization
b. Southern blot analysis
c. DNA sequencing
d. Northern blot analysis

Ans. d. Northern blot analysis

Methods to detect point mutation

Method	Type of Mutation Detected
Southern blot	Large deletion, insertion, rearrangement, expansions of triplet repeat, amplification
DNA sequencing	Point mutations, small deletions and insertions
Restriction fragment polymorphism (RFLP)	Point mutations, small deletions and insertions
Single-strand conformational polymorphism (SSCP)	Point mutations, small deletions and insertions
Denaturing gradient gel electrophoresis (DGGE)	Point mutations, small deletions and insertions
RNAse cleavage	Point mutations, small deletions and insertions
Oligonucleotide specific hybridization (OSH)	Point mutations, small deletions and insertions
Microarrays	Point mutations, small deletions and insertions Genotyping of SNPs

44. DNA fingerprinting is based on possessing in DNA of: (PGI Dec 08)

a. Constant Tandem Repeat
b. Variable Number Tandem Repeat
c. Non-repetitive sequence
d. Exon
e. Intron in eukaryotes

Ans. b. Variable Number Tandem Repeat

DNA Fingerprinting

- The use of normal genetic variation in the DNA (SNP or VNTR or RFLP) to establish a unique pattern of DNA fragments for an individual.
- This is also called DNA Profiling.
- Most commonly used is VNTR or repeat length polymorphism.
- The process of DNA fingerprinting was invented by Alec Jeffreys in 1985.
- Primer is needed.

45. RFLP, true is/are: (PGI Dec 06)

a. Detects mutation
b. Recognizes trinucleotide repeats
c. Detects deletion
d. Blunt ends are produced
e. Always short ends are produced

Ans. a, c, d. Detects mutation, Detects deletion, Blunt ends are produced *(Ref: Harper 30/e page 463)*

Two type of DNA Variations that result in RFLP are:

1. Single Nucleotide Polymorphism (SNP)
2. Variable Number Tandem Repeat (VNTR)

Uses of RFLP

- Tracing chromosomes from parent to offspring.
- Prenatal diagnosis of diseases
  - Direct diagnosis of sickle cell disease using RFLP
  - Indirect, prenatal diagnosis of phenylketonuria
- Medicolegal uses
- Detect mutations, point mutation, insertion, deletion.

Gene Therapy

46. Natural methods of horizontal gene transfer in bacteria: (PGI Nov 2009)

a. Transformation
b. Transduction
c. Conjugation
d. Electroporation
e. Mutation

Ans. a, b, c. Transformation, Transduction, Conjugation

- Electroporation is an artificial method of gene delivery
- Trinucleotide ..., X chromosome ...

47. Methods of introducing gene in target cells are all except: (AIIMS Nov 2010)

a. Electroporation
b. Transfection
c. Site directed recombination
d. FISH

Ans. d. FISH

(Ref: lib.store.yahoo.net/.../How-to-Choose-the-Optimal-Gene-Delivery-Method.pdf)

Electroporation

Electroporation is a method of introducing nucleic acids into cells by exposing the cells to a rapid pulse of high-voltage current, causing pores in the cell membrane to open temporarily.

This allows exogenous DNA to pass through the pores and into the cytoplasm of the cells.

Typically, the gene transfer efficiency is relatively low, and electroporation frequently results in a high incidence of cell death.

Transfection: Introduction of gene through nonviral vectors.

Transduction: Introduction of gene through viral vectors.

Site directed recombination: The method by which endogenous gene is replaced by passenger gene by homologous recombination.

Fluorescent In situ Hybridization (FISH)–Not a method of gene delivery.

Gene Therapy

48. The first gene therapy (somatic enzyme) was successfully done in: (PGI Dec 07)

a. SCID
b. Phenylketonuria
c. Thalassemia
d. Cystic fibrosis
e. Alkaptonuria

Ans. a. SCID

Dr French Anderson did the first gene therapy to treat SCID.

49. Gene therapy methods are: (PGI June 03)

a. Electroporation
b. Intranuclear injection
c. Site directed mutagenesis
d. Retrovirus

Ans. a, b, c, d. Electroporation, Intranuclear injection, Site directed mutagenesis, Retrovirus.

50. Purpose of gene therapy: (PGI June 03)

a. Replacement of abnormal gene by normal gene
b. Replacement of normal gene by abnormal gene
c. Knock out of abnormal gene
d. Introduction of viral gene

Ans. a. Replacement of abnormal gene by normal gene.

Novel area of therapeutics

Gene Therapy

Intracellular delivery of genes to generate a therapeutic effect by correcting an existing abnormality

Divided into:

1. Somatic cell gene therapy—Gene is introduced into somatic cells.
2. Germ cell gene therapy (Transgenic animal).

Transgenic animals

51. RNAi in gene expression denotes

a. Knockdown **(AIIMS May 2015)**
b. Knock up

c. Knock in
d. Knock out

Ans. a. Knock down *(Ref: Harper 30/e page 447)*

Gene Knock out: Endogenous gene is replaced by mutated transgene by homologous recombination in embryonic stem cell

Gene Knock in: Mutated endogenous gene is replaced by normal transgene by homologous recombination in embryonic stem cell

Gene Knock down: siRNA or miRNA induced gene silencing called RNA interference or RNAi

Gene Knock up: Using transcription factors, transcription of gene is increased.

52. True statement about transgenic mice: **(PGI Nov 2010)**

a. Developed from DNA insertion into fertilized egg
b. Have same genome as parents except one or more genes
c. Identical genome to parent mice
d. Produced by breeding over several generations
e. Homozygous are selected

Ans. a, b, d, e. Developed from DNA insertion into fertilized egg, Have same genome as parents except one or more genes, Produced by breeding over several generations. Homozygous are selected

(Textbook of biochemistry Thomas M Devlin 7/e page 292, 293)

Different Strategies of Genetic Modification

Injection of the transgene into the **male pronucleus of fertilized ovum**.

Steps for generation of a Knock out Mouse

- Inactivating a recombinant purified gene
- Culture embryonic stem (ES) cell from mice
- Transfection of ES with cloned mutated non-functional gene
- A few cultured embryonic cells contain the non-functional gene through homologous recombination
- Isolate ES cells with altered gene
- Microinjection of altered ES cells into mouse blastocyst
- Implantation of blastocyst into foster mother
- Breed to many generation till offspring with altered gene in its germ cell
- Homogygous are selected.

53. The function of a gene is determined by: **(AI 2012)**

a. Southern blot
b. Western blot
c. Inserting in transgenic mice
d. Inserting as a knock out gene

Ans. d> c. Inserting as a knock out gene > Inserting in transgenic mice. *(Ref: Text book of biochemistry Thomas M Devlin 7/e page 292, 293)*

- **Inserting in transgenic mice site specific integration is not possible.**
- **Knock out transgenesis is by homologous recombination. So site specific integration possible. So d>c.**

Bioinformatics

54. The following are used to study pathological genome except:

a. Genbank
b. Entrez gene
c. Hapmap
d. BLAST

Ans. d. BLAST *(Ref: Harper 30/e page 98, 99)*

Bioinformatic and Genomic Resources

The large collection of databases that have been developed for the assembly, annotation, analysis and distribution of biological and biomedical data reflects the breadth and variety of contemporary molecular, biochemical, epidemiological, and clinical research. The prominent bioinformatics resources: UniProt, GenBank, and the Protein Database (PDB) represent three of the oldest and most widely used bioinformatics databases.

Uniprot: The world's most comprehensive resource on **protein structure and function**

Genbank: The store all known biological **nucleotide sequences** and their translations in a searchable form

PDB (Protein Data Base) a repository of the **three-dimensional structures of proteins**, polynucleotides, and other biological macromolecules

HapMap is a comprehensive effort to identify SNPs associated with common human diseases and differential responses to pharmaceuticals.

ENCODE (Encyclopedia of DNA Elements) Project-Identification of all thefunctional elements of the genome will vastly expand our understanding of the molecular events that underlie human development, health, and disease.

Entrez **Gene** provides a variety of **information about individual human genes**. The information includes the sequence of the genome in and around the gene, exon-intron boundaries, the sequence of the mRNA(s) produced from the gene, and any known phenotypes associated with a given mutation of the gene in question.

dbGAP (Database of Genotype and Phenotype), compiles the results of research into the links between specific genotypes and phenotypes

BLAST (Basic Local Alignment Search Tool) is a method to identify protein by homology. This is not a bioinformatic data base.

55. Study of structure and products of gene is: (PGI Dec 05)

a. Genomics
b. Proteomics
c. Bioinformatics
d. Cytogenetics
e. Pharmacogenomics

Ans. a. Genomics *(Ref: Harper 30/e page 98, 99)*

- Bioinformatics: The discipline concerned with the collection, storage, and analysis of biologic data, mainly DNA and protein sequences
- Genome: The complete set of genes of an organism
- Genomics: In depth study of the structures and functions of genomes
- Transcriptome: The complete set of RNA transcripts produced by the genome during a fixed period of time.
- Transcriptomics: The comprehensive study of transcriptome
- Proteome: The complete complement of proteins of an organism
- Proteomics: The systematic study of structures and functions of proteomes and their variation in health and disease
- Exome: The nucleotide sequence of the entire complement of mRNA exons expressed in a particular cell, tissue, organ or organism.

56. Study of multiplication of proteins in disease process is called: (AI 07)

a. Proteomics
b. Genomics
c. Glycomics
d. Nucleomics

Ans. a. Proteomics *(Ref: Harper 30/e page 98, 99)*

57. What biologist uses to diagnose and treat diseases with disorders with multigenic inheritance?

a. Gene card
b. Tag SNPs
c. Flipped card
d. Virtual Cell

Ans. b. Tag SNPs *(Ref: Robbins 9/e page 179)*

Linkage and Association Studies

There are two primary strategies for mapping genes that cause or increase susceptibility to human disease:

1. Classic linkage can be performed based on a known genetic model or, when the model is unknown, by studying pairs of affected relatives
2. Disease genes can be mapped using allelic association studies (Genome Wide Association Studies, GWAS)

Genome-wide association studies (GWAS) have elucidated numerous disease-associated loci and are providing novel insights into the allelic architecture of complex traits. These studies have been facilitated by the availability of comprehensive catalogues of human single-nucleotide polymorphism (SNP) haplotypes generated through the HapMap Project.

The data generated by the HapMap Project are greatly facilitating GWAS for the characterization of complex disorders. Adjacent SNPs are inherited together as blocks, and these blocks can be identified by genotyping selected marker SNPs, so-called Tag SNPs, thereby reducing cost and workload.

58. Which of the following statement is true about Linkage analysis? (AIIMS Nov 07)

a. Detection of characteristic DNA polymorphism in a family associated with disorders
b. Useful to make pedigree chart to show affected and non-affected family members
c. Used to make a pedigree chart to show non-paternity
d. Nongene mapping method of genetic study

Ans. a. Detection of characteristic DNA polymorphism in a family associated with disorders

(Ref: Robbins 9/e page 177-179)

Linkage analysis

- The genetic marker loci very close to the disease allele, that are transmitted through pedigrees called linkage disequilibrium.
- Assess the genetic marker loci in **family members** having the disease or trait of interest
- With time it becomes possible to define a panel of marker loci, all of which co-segregate with the putative diseases
- Hence, Linkage analysis facilitates localization and cloning of the disease allele.
- The genetic markers used are, SNPs and repeat-length polymorphisms (minisatellite and microsatellite repeats).

5

Section | Miscellaneous

CHAPTERS

15. Vitamins and Minerals
16. Heme Metabolism and Hemoglobins
17. TCA Cycle and Biological Oxidation
18. Free Radicals, Xenobiotics and Metabolism of Alcohol

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15 Vitamins and Minerals

Topics Included

- Fat Soluble Vitamins
- Water Soluble Vitamins
- Minerals

VITAMINS

Definition

Vitamins are organic compounds occurring in small quantities in different natural foods and necessary for growth and maintenance of good health.

Vitamins are mainly classified into

- *Fat soluble vitamins:* Vitamins A, D, E and K
- *Water soluble vitamins:* B Complex Vitamins and Vitamin C.

Endogenously synthesized Vitamins

Vitamins are generally not synthesized by the humans, but some vitamins can be synthesized endogenously. They are:

- Vitamin D from precursor steroids
- Vitamin K, Biotin, and pantothenic acid by the intestinal microflora
- Niacin from tryptophan, an essential amino acid.

Fat Soluble Vitamins

Vitamin A

- Ring structure present in Vitamin A is β ionone ring
- Provitamin A, β carotene contain 2 β ionone ring
- Cleaved in the intestine by a **dioxygenase**.

Retinoids

All compounds chemically related to retinol are called retinoids. They are:

- *Retinal:* **11 cis retinal** for normal vision
- *Retinoic acid:* Normal morphogenesis, growth and cell differentiation
- *Retinol:* Reproduction.

Vitamin A, in the strictest sense, refers to Retinol

Carotenoids

- They are provitamins of Vitamin A present in plants
- More than 600 carotenoids in nature, and approximately 50 of them can be metabolized to vitamin A
- β Carotene is the most prevalent carotenoid in the food supply that has provitamin A activity.

Nonprovitamin A Carotenoids

- *Lutein and Zeaxanthin:* Protect against macular degeneration
- *Lycopene*ϙ*:* Protect against prostate cancer.

Vitamin A Metabolism

Absorption and transport of Vitamin A

- Beta Carotene from plant sources is absorbed and cleaved to two molecules of Retinal by Beta Carotene Dioxygenase. Retinal is reduced to retinol by Retinol Reductase
- Retinol ester from animal sources is hydrolyzed in the intestinal lumen to Retinol and absorbed into the intestinal cells
- Retinol from animal and plant sources is reesterified to retinol esters and transported in Chylomicronsϙ to Liver

– Uptake takes place in liver cells by means of **apo E receptors.**

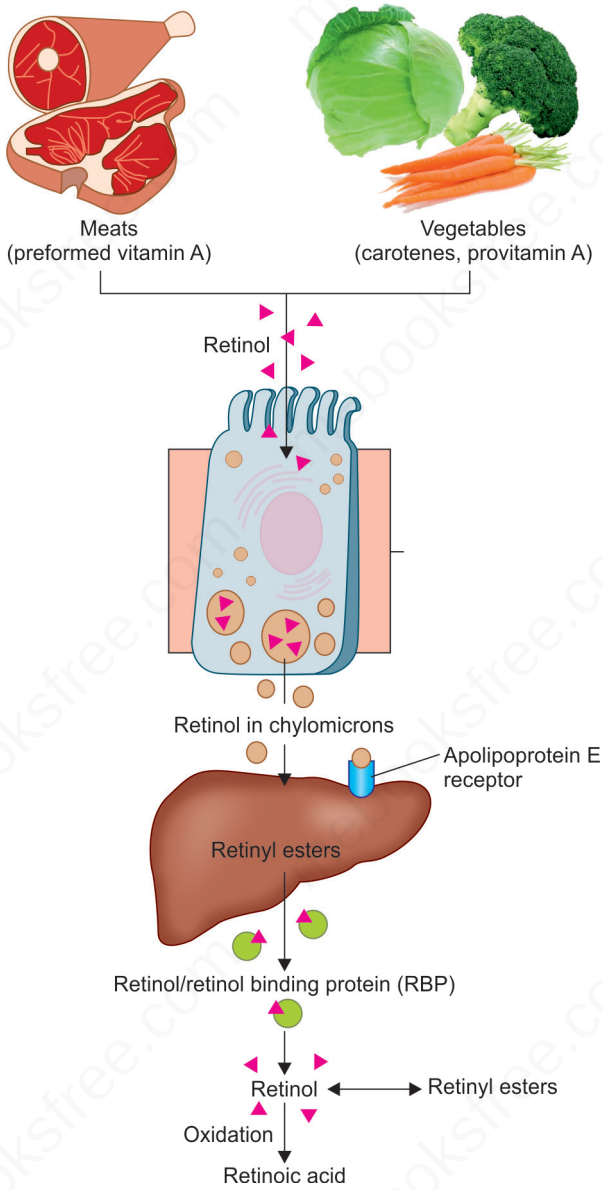


Fig. 15.1: Metabolism of Vitamin A

Storage of Vitamin A

Stored in the **Liver Perisinusoidal Stellate (Ito) cells** as Retinyl Ester (Retinol Palmitate).

Transport of Vitamin A from Liver to Target Organs

Carried to target sites in the plasma as trimolecular complex bound to Retinol Binding Protein (RBP) and Transthyretin.

Functions of Vitamin A

- **Vision**
  - Visual process involve 3 forms of Vitamin A containing pigments
  - ***Rhodopsin***
    - Most light sensitive pigment present in rods
    - Formed by covalent association between **11 cis retinal** and 7-transmembrane rod protein called opsin.

Three iodopsin each responsive to specific colors in cones in bright light.

- **Regulation of gene expression and differentiation**
  - Retinoic Acids are involved in this function
  - Biologically important retinoic acids are all Trans-retinoic acid and 9 -cis retinoic acid
  - They act like steroid hormones
  - They bind to **nuclear receptors**.

Retinoic acid receptors

Retinoid receptors regulate transcription by binding to specific DNA site.

- Retinoic Acid Receptors (RARs) binds with high affinity to all: Transretinoic acid and 9 cis retinoic acid
- Retinoic X receptor (RXRs) binds only to 9 cis retinoic acid
- **Normal reproduction**
  - Retinol is necessary for this function.
- Maintenance of normal epithelium of skin and mucosa
- **Antioxidant Properties and photo protective property is attributed to Beta Carotenes**
- **Host resistance to infection.**

Vitamin A deficiency manifestations

- Most common vitamin deficiency
- Most common cause of preventable blindness
- **Eyes**
  - Loss of sensitivity to green light is the earliest manifestation
  - All the ocular manifestations are collectively called as Xerophthalmia
  - Impairment to adapt in dim light, i.e. night blindness or Nyctalopia is the earliest symptom
  - Conjunctival Xerosis (Dryness of Conjunctiva)
  - Bitot's spots (white patches of keratinized epithelium appearing on the sclera)

- Blinding corneal ulceration and necrosis
- Keratomalacia (softening of the cornea)
- Corneal scarring that causes blindness.

- **Skin and Mucosa**
  - **Epithelial metaplasia and keratinization**
  - Hyperplasia and hyperkeratinization of the epidermis with plugging of ducts of adnexal gland produce **Follicular Hyperkeratosis**[Q] or Papular dermatosis. This is called as **Phrynoderma** or Toad Skin
  - Squamous Metaplasia in the mucus secreting epithelium of upper respiratory tract and urinary tract
  - Loss of taste sensation.

Remember
- Concurrent Zinc deficiency can interfere with mobilization of Vitamin A from liver stores.
- Alcohol interferes with conversion of retinol to retinaldehyde in the eyes.

Vitamin A as therapeutic agent
- β Carotene used in cutaneous Porphyria
- All transretinoic acid in acute Promyelocytic Leukemia [called as differentiation therapy]
- 13 cis retinoic acid [Isotretinoin] in cystic Acne
- 13 cis retinoic acid in childhood neuroblastoma.

Hypervitaminosis A
- Common in arctic explorers who eat polar bear liver.
- Organelle damaged in hypervitaminosis is **Lysosomes**
- Acute toxicity: Pseudotumor cerebri[Q] (headache, dizziness, vomiting, stupor, and blurred vision, symptoms that may be confused with a brain tumor) and exfoliative dermatitis. In the liver, hepatomegaly and hyperlipidemia
- Chronic toxicity: If intake of > 50,000 IU/day for > 3 months
- Weight loss, anorexia, nausea, vomiting, bony exostosis, bone and joint pain, decreased cognition, hepatomegaly progresses to cirrhosis
- ***Retinoic acid stimulates osteoclast production and activity leading to increased bone resorption and high risk of fractures, especially hip fractures***
- ***In pregnancy retinoids causes teratogenic effects.***

Carotenemia
- Persistent excessive consumption of foods rich in Carotenoids
- Causes yellow staining of skin but not sclera (Unlike Hyperbilirubinemia which stain both skin and sclera).

Required Daily Allowance of Vitamin A[Q] (µg of Retinol) (ICMR 2010)

Children (1–6 yrs)	400 µg/day
Men	600 µg/day
Women	600 µg/day
Pregnancy	800 µg/day
Lactation	950 µg/day

Units of Vitamin A
- Vitamin A in food is expressed as micrograms of retinol equivalent
- 6 µg of beta Carotene = 1 µg of preformed retinol
- Pure Vitamin A for pharmaceutical uses is expressed International Units (IU) 1 IU = 0.3 µg of Retinol
- 1 µg of Retinol = 3.33 IU
- In 2001 USA Canadian Dietary Reference value introduced the term Retinol Activity Equivalent (RAE) 1 RAE = 1 µg of Retinol or 12 µg of Beta carotene.

Sources of Vitamin A
- Animal food (mainly as Retinol)
- Plant food as Carotenes.

Animal sources
- Fish liver oils[Q] are the rich sources of Vitamin A
- **Halibut liver oil** is the richest source (900000 µg/100 g) followed by cod liver oil
- Other animal sources are liver, egg, butter, cheese, whole milk, fish and meat.

Plant sources
- Richest plant source is **Carrot**
- Others are GLV like Spinach, Amaranth, Green and yellow fruits like papaya, mango, pumpkin.

Treatment of Vitamin A deficiency
- 200000 IU or 110 mg of Retinol Palmitate orally in two successive days.

Prevention of Vitamin A deficiency
- Single massive dose 200000 IU to children (1–6 years) once in 6 months
- Single massive dose 100000 IU to children (6 mo–1 year) once in 6 months.

Assay of vitamin A
- Dark adaptation time
- Serum Vitamin A by **Carr and Price reaction.**

Vitamin D

Group of sterols having a hormone like function

- *Ergocalciferol (Vit D2):* Commercial Vitamin D obtained from the fungus, ergot
- *Cholecalciferol (Vit D3):* Endogenous synthesis from 7 Dehydrocholesterol.

Vitamin D metabolism

- Sources of Vitamin D
  - The major source of vitamin D for humans is its **endogenous synthesis** in the skin by photochemical conversion of a precursor, **7-dehydrocholesterol,** to Cholecalciferol or Vitamin D3 via the energy of solar or artificial **UV light in the range of 290 to 315 nm (UVB radiation)** in the **stratum corneum of the epidermis** of skin
  - Absorption of vitamin D from foods and supplements in the gut
- Binding of vitamin D from both of these sources to plasma **α1-globulin (D-binding protein or DBP)** and transport into the liver.

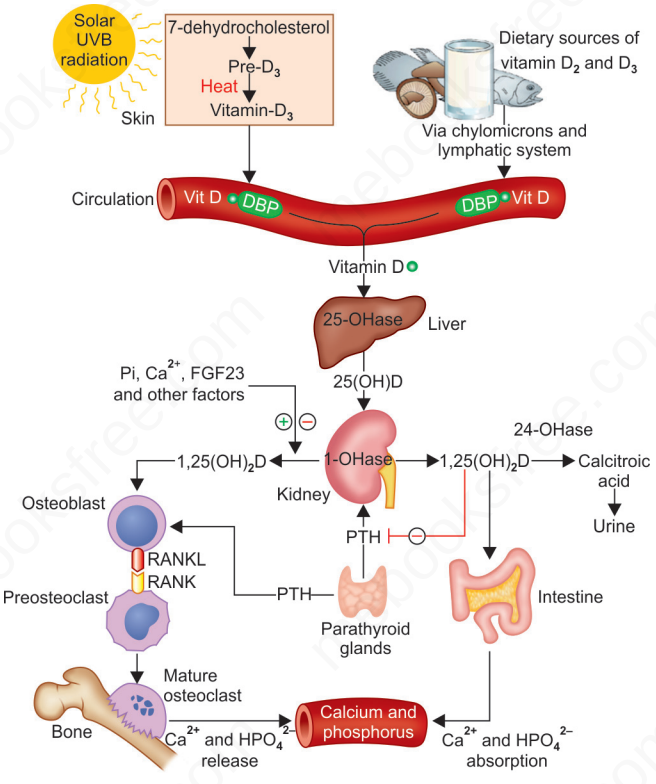


Fig. 15.2: Metabolism of Vitamin D

- Conversion of vitamin D into 25-hydroxy cholecalciferol (25-OH-D) in the liver, through the effect of **25- Hydroxylases.** Most abundant circulatory form of Vitamin D. This is because there is little regulation of this liver hydroxylation. The measurement of 25-OH D is the standard method for determining patients' Vitamin D status
- Conversion of 25-OH-D into **1, 25-dihydroxy vitamin D, (1, 25 $(OH)_2$D3) or Calcitriol** in the **kidney**Q**, the biologically most active**Q **form of vitamin D,** through the activity of **α1-hydroxylase. This is the rate limiting step. PTH and Hypophosphatemia** upregulate 1 α Hydroxylase. Hyperphosphatemia and 1, 25 OH D inhibit the enzyme
- When Ca_2^+ level is high, kidney produces the relatively inactive metabolite 24, 25 Dihydroxy Cholecalciferol (Calcitroic acid) excreted through urine.

Functions of Vitamin D

Regulation of calcium and phosphorus homeostasis

Action on intestine

- Vitamin D increases Ca^{2+} absorption
- By increasing the transcription of **TRPV6** (a member of the transient receptor potential vanilloid family), which encodes a critical calcium transport channel. This increases Calcium absorption from duodenum.

Action on kidney

- Vitamin D increases Ca^{2+} and Phosphorus reabsorption
- Increases calcium influx in distal tubules of the kidney through the increased expression of **TRPV5**, another member of the transient receptor potential vanilloid family.

Action on bones

- 1, 25-dihydroxy vitamin D and parathyroid hormone enhance the expression of RANKL (receptor activator of NF-κB ligand) on osteoblasts
- **RANKL** binds to its receptor (RANK) located in preosteoclasts, inducing the differentiation of these cells into mature osteoclasts
- They dissolve bone and release calcium and phosphorus into the circulation.

Immunomodulatory and antiproliferative effects

- Prevent infection by **Mycobacterium tuberculosis**
- Within macrophages, synthesis of 1, 25-dihydroxy-vitamin D occurs through the activity of CYP27B located in the mitochondria
- Pathogen-induced activation of Toll-like receptors in macrophages causes a transcription-induced increase in vitamin D receptor and CYP27B.

- The resultant production of 1, 25-dihydroxy vitamin D then stimulates the synthesis **of cathelicidin**, an antimicrobial peptide from the defensin family, which is effective against infection by Mycobacterium tuberculosis.

Antiproliferative role of Vitamin D

1, 25 $(OH)_2$ D level less than 20 ng/mL is associated with increase in incidence of

- Colon cancer
- Breast cancer
- Prostate cancer

Mineralization of bones

- Vitamin D contributes to mineralization of osteoid matrix and epiphyseal cartilage in both flat and long bones
- It stimulates osteoblast to synthesize calcium binding protein osteocalcin involved in deposition of calcium during bone development.

Vitamin D deficiency

- The normal reference range for circulating 25-(OH) D is 20 to 100 ng/mL
- The concentration circulating 25-(OH) D < 20 ng/mL is called Vitamin D deficiency.

Causes inadequate mineralization of bone osteoid

- Before closure of epiphysis: **Rickets in children**
- After closure of epiphysis: **Osteomalacia in adults.**

Biochemical defect of different types of rickets

Nutritional Vitamin D Deficiency

Most common cause of rickets globally.

Concept of biochemical changes that occur in nutritional Vitamin D deficiency

- Due to Vitamin D deficiency, Serum Calcium level and Phosphorus level is low
- This causes Secondary Hyperparathyroidism, so PTH level is high
- This increases the 1 α hydroxylation in kidney, so 1,25 D level increases
- This will increase the Serum Calcium level, but Phosphorus level remain at low level
- So, Serum Calcium level is variable, Serum Phosphorus is low, S PTH increase, 25 D is decreased,1,25 D is low initially but later increase due to secondary hyperparathyroidism.

Remember

- Serum calcium need not be always low in Rickets
- 1,25 D level also need not be always low in Rickets
- Serum Phosphorus remain low.

Vitamin D–dependent rickets type 1 (Pseudo-vitamin D–resistant rickets)

- An autosomal recessive disorder
- Mutations in the gene encoding renal 1α-hydroxylase
- Prevent conversion of 25 D to 1,25 D
- Even with high PTH, as 1 α Hydroxylase is defective, 1,25 D is low
- Usually presents in first 2 years of life
- With classic features of rickets.

Concept of biochemical changes in Vitamin D Dependent Rickets Type I

- *Inspite of secondary hyperparathyroidism,*1,25 D will remain decreased as 1 α hydroxylase gene is mutated.

Vitamin D–dependent rickets type 2 (True vitamin D–resistant rickets)

- An autosomal recessive disorder
- Due to mutations in the gene encoding the vitamin D receptor causing end-organ resistance to the active metabolite 1,25 D
- Presents in infancy with less severe manifestation
- 50–70% of children have alopecia
- Epidermal cyst is also a common manifestation.

X-linked hypophosphatemic rickets

- X-linked dominant disorder
- The most common hypophosphatemic rickets
- The defective gene is called *PHEX* (**PH**osphate-regulating gene with homology to ***E***ndopeptidases on the ***X*** chromosome)
- The product of this gene have either a direct or an indirect role in inactivating a phosphatonin or phosphatonins (FGF-23)
- Mutation of PHEX gene lead to increased level of FGF-23
- Hypophosphatemia with normal PTH, normal calcium and low or inappropriately normal 1,25 D are the lab findings.

Phosphatonins (FGF-23)

- Humoral mediator that decrease renal tubular reabsorption of phosphate, therefore decreases serum phosphorus
- This also decreases the activity of 1 α hydroxylase, resulting in deficiency of 1,25 D
- Fibroblast Growth Factor-23 (FGF-23) is the most well characterized phosphatonin
- Increased level of phosphatonins causes increased excretion of phosphorus in urine
- So serum Phosphorus is decreased.

Autosomal dominant hypophosphatemic rickets

- An autosomal dominant condition

- Due to a mutation in the gene encoding FGF-23 which prevents the degradation of FGF-23 by proteases. So there is increased levels of phosphatonins
  - Hypophosphatemia with normal PTH, normal calcium and low or inappropriately normal 1,25 D are the lab findings.

Remember
- Biochemical findings of X linked and autosomal dominant Hypophosphatemic rickets is same as phosphatonins is excess in both
- Hypophosphatemia is due to increased excretion of phosphates through kidney by phosphatonins
- Low or normal 1,25D is due to decreased activity of 1 α Hydroxylase.

Autosomal Recessive Hypophosphatemic rickets

- Extremely rare disorder due to mutation in the gene encoding dentin matrix protein 1, which results in elevated level of FGF-23.

Hereditary Hypophosphatemic rickets with hypercalciuria (HHRH)

- Autosomal recessive disorder due to mutation in the gene for a sodium phosphate cotransporter in the proximal renal tubules
- Hypophosphatemia, stimulates production of 1,25 D
- This causes increased intestinal absorption of calcium
- Symptoms of rickets, along with muscle pain, bone pain short stature with disproportionate decrease in length of lower extremities, kidney stones.

Chronic Renal Failure

- There is decreased activity of 1α-hydroxylase in the kidney, leading to diminished production of 1,25-D.
- Unlike the other causes of vitamin D deficiency, patients have **hyperphosphatemia** as a result of decreased renal excretion.

Conditions causing over production of phosphatonins which causes rachitic findings
- Tumor-induced osteomalacia
- McCune-Albright Syndrome (entity that has triad of Polyostotic fibrous dysplasia, Hyperpigmented macules, polyendocrinopoathy)
- Epidermal nevus Syndrome
- Neurofibromatosis in children.

Requirement of Vitamin D

- Children: 10 μg/day (400 IU)
- Adults: 5 μg/day (200 IU)
- Pregnancy, Lactation: 10 μg/day (400 IU).

Vitamin D is toxic in excess

- Upper limit of Vitamin D intake has been set 4000 IU/day
- Some *infants are sensitive to intakes of vitamin D as low as 50 μg/day*°, resulting in an elevated plasma concentration of calcium
- This can lead to contraction of blood vessels, high bloodpressure, and **calcinosis**—the calcification of soft tissues
- Although excess dietary vitamin D is toxic, excessive exposure to sunlight does not lead to vitamin D poisoning, because there is a limited capacity to form the precursor, 7-dehydrocholesterol, and prolonged exposure of previtamin D to sunlight leads to formation of inactive compounds.

Beneficial effects of Vitamin D
- Protective against the cancer of Prostate, Colorectal cancer
- Protective against Prediabetes, and metabolic Syndrome.

Laboratory findings in disorders causing rickets

Disorder	Serum Calcium	S Phosphorus	PTH	25 (OH)D	1,25 (OH)D	ALP
Vitamin D Deficiency	N/Decrease	Decrease	Increase	Decrease	Decrease, N, Increase	Increase
Vitamin D Dependent Rickets Type I	N/Decrease	Decrease	Increase	N	Decrease	Increase
Vitamin D Dependent Rickets TypeII	N/Decrease	Decrease	Increase	N	Increased	Increase
Chronic renal Failure	N/Decrease	Increase	Increase	N	Decrease	Increase
X Linked Hypophosphatemic Rickets	N	Decrease	Normal	N	Relatively Decrease	Increase
Autosomal Dominant Hypophosphatemic Rickets	N	Decrease	Normal	N	Relatively Decreased	Increase

Sources of Vitamin D

- Sunlight
- **Foods: Only animal sources Liver, Egg yolk, butter and liver oils. Out of the food sources Fish liver oils are the richest source**
- The richest source of Vitamin D is also Halibut Liver oil.

Assay of Vitamin D

- The release into the circulation of osteocalcin provides an index of vitamin D status
- 25(OH) Vitamin D level is measured in the serum indicate Vitamin D status.

Vitamin E

- *Vitamin E is a collective name for all stereoisomers of* tocopherols and tocotrienols
- The most powerful naturally occurring antioxidant[Q].

Ring Structure present in Vitamin E

- Chromane (Tocol) ring with isoprenoid side chain
- **Vitamin E is carried to liver in Chylomicron.**

Biochemical functions of Vitamin E

- Biologically most potent form of Vitamin E is **α Tocopherol**[Q]
- **Chain-breaking antioxidant**[Q] and is an efficient pyroxyl radical scavenger that protects low-density lipoproteins (LDLs) and polyunsaturated fats in membranes from oxidation
- Lipid soluble antioxidant.

Relationship with Selenium

- Selenium decrease the requirement of Vitamin E[o].

Vitamin E deficiency

- **Axonal degeneration** and of the large myelinated axons and result in posterior column and spinocerebellar symptoms
- **Hemolytic anemia:** The erythrocyte membranes are abnormally fragile as a result of poor lipid peroxidation, leading to hemolytic anemia
- **Peripheral neuropathy** initially characterised by Areflexia with progression to ataxic gait, decreased position and vibration sense
- **Spinocerebellar ataxia**
- Skeletal myopathy
- Pigmented retinopathy
- Ophthalmoplegia.

Vitamin E in high doses may protect against

- Oxygen-induced retrolental fibroplasia
- Bronchopulmonary dysplasia
- Intraventricular hemorrhage of prematurity
- Treat intermittent claudication
- Slow the aging process.

Toxicity of Vitamin E

- Reduce platelet aggregation and interfere with Vitamin K.

Required daily allowance

- Males 10 mg/day
- Females 8 mg/day
- Pregnancy 10 mg/day
- Lactation 12 mg/day.

Sources of Vitamin E

Vegetable oils like Wheat germ oil, sunflower oil, Cotton seed oil, etc.

Vitamin K

- Naphthoquinone derivative with long isoprenoid side chain
- ***Letter K is the abbreviation of German word, Koagulation Vitamin.***

Three forms of Vitamin K

- Vitamin K1: Phylloquinone from dietary sources
- Vitamin K2: **Menaquinone** Synthesized by Bacterial Flora
- Vitamin K3: **Menadione** (and Menadiol diacetate): Synthetic, **Water Soluble**.

Functions of Vitamin K

Vitamin K is required for the post-translational carboxylation of glutamic acid (Gamma Carboxylation), which is necessary for calcium binding to γ carboxylated proteins.

- Prothrombin (factor II)
- Factors VII, IX, and X
- Protein C, protein S
- Proteins found in bone (osteocalcin)
- Matrix Gla protein
- Nephrocalcin in kidney
- Product of growth arrest specific gene Gas6.

Drugs causing Vitamin K deficiency

Warfarin and Dicoumoral inhibit γ carboxylation by competitively inhibiting the enzyme that convert vitamin K to its active hydroquinone form

- Antiobesity drug orlistat.

Vitamin K Deficiency

- Elevated prothrombin time, bleeding time

- Newborns, especially premature infants are particularly susceptible to Vitamin K deficiency because of low fat stores, low breast milk levels of vitamin K, sterility of the infantile intestinal tract, liver immaturity, and poor placental transport.

Hypervitaminosis K

- Hemolysis
- Hyperbilirubinemia
- Kernicterus and brain damage.

Water Soluble Vitamins

- B Complex Vitamins
- **Vitamin C**

Thiamin (Vitamin B1)

- **Thiamin is also called Aneurine**

Sources

- Aleurone layer of cereals. Hence whole wheat flour and unpolished hand pound rice has better nutritive value. Yeast is also a good source of thiamine.

Active form of Thiamin

Thiamine Pyrophosphate (TPP) also called Thiamine diphosphate (TDP).

Thiamine and nerve conduction

Thiamin triphosphate has a role in nerve conduction; it phosphorylates, and so activates, a chloride channel in the nerve membrane.

Coenzyme Role of Thiamine Pyrophosphate[Q]

Thiamine generally function in the decarboxylation reaction of alpha keto acids and branched chain amino acids

- **Pyruvate Dehydrogenase**[Q] which convert Pyruvate to Acetyl CoA
- **αKetoGlutarate Dehydrogenase**[Q] in Citric Acid Cycle which convert α KetoGlutarate to Succinyl CoA
- **Branched Chain Ketoacid Dehydrogenase**[Q] which catalyses oxidative decarboxylation of Branched Chain Amino acids
- **Trans Ketolase**[Q] in **Pentose Phosphate Pathway**[Q]. This is the biochemical basis of assay of Thiamine status of the body.

Deficiency of Vitamin B1 (Thiamin)

BeriBeri[Q]

Two types

1. **Wet beriberi**: Marked peripheral vasodilatation, resulting in high output cardiac failure with dyspnoea, tachycardia, cardiomegaly, pulmonary and peripheral edema.
2. **Dry beriberi**: Involves both peripheral and central nervous system.

Peripheral nervous system

- Typically a symmetric motor and sensory neuropathy with pain, paraesthesia and loss of reflexes. The legs are affected more than the arms.

Central nervous system

Wernicke's Encephalopathy–in alcoholics with chronic Thiamine deficiency

- Horizontal Nystagmus
- Ophthalmoplegia
- Truncal ataxia
- Confusion
- Wernicke- Korsakoff Syndrome
- Along with features of Wernicke's Encephalopathy
- Amnesia
- Confabulatory psychosis.

Acute pernicious (fulminating) beriberi (shoshin beriberi), in which heart failure and metabolic abnormalities predominate.

Biochemical assessment of thiamin deficiency

- Erythrocyte Transketolase activity is reduced
- Urinary Thiamine excretion.

Thiamin toxicity

- There is no known toxicity of thiamine

Recommended Daily Allowance (RDA) of Vitamin B1

- 1–1.5 mg/day.

Riboflavin (Vitamin B2)

- Is called Warburg Yellow enzyme[Q] of cellular respiration
- **Riboflavin is heat stable**
- Enzymes containing riboflavin are called Flavoproteins
- Act as respiratory coenzyme and an electron donor.

Active forms of Riboflavin

- ***They are FAD (Flavin Adenine Dinucleotide) and FMN (Flavin Mononucleotide)***

Coenzyme Role of Riboflavin

FMN Dependent Enzymes[Q]

- L- Amino Acid Oxidase
- NADH Dehydrogenase (Complex I of ETC)
- Monoamino Oxidase

FAD Dependent Enzymes

- Complex II (Succinate Dehydrogenase) of ETC
- D Amino Acid Oxidase
- Acyl CoA Dehydrogenase

Contd...

Contd...

- Alpha Ketoglutarate Dehydrogenase
- Pyruvate Dehydrogenase
- Xanthine Oxidase.

Deficiency manifestation of Vitamin B2 (Riboflavin)

Magenta tongue (Glossitis), angular stomatitis, **Seborrheic Dermatitis**, Cheilosis, **Corneal vascularization**, anemia

Biochemical Assessment of Nutritional status of Riboflavin

- Measurement of activation of erythrocyte Glutathione Reductase by FAD added in vitro
- Urinary excretion of Riboflavin.

Riboflavin toxicity

- Riboflavin toxicity is not reported yet because of limited absorption capacity of GIT.

RDA of Riboflavin

- 1.5 mg/day.

Niacin or Nicotinic Acid (Vitamin B3)

- Not strictly a Vitamin
- Can be synthesized from Tryptophan
- 60 mg of Tryptophan yield 1 mg of Niacin.

Active form of niacin

- Two Coenzyme forms are NAD+(Nicotinamide Adenine Dinucleotide) and NADP+(Nicotinamide Adenine Dinucleotide Phosphate).

Coenzyme Role of Niacin

- Important in numerous oxidation reduction reactions.

NAD+ linked Enzymes

- Lactate Dehydrogenase
- Pyruvate Dehydrogenase
- αKetoGlutarate Dehydrogenase
- Isocitrate Dehydrogenase
- Malate Dehydrogenase
- βHydroxy Acyl CoA Dehydrogenase
- Glycerol 3 Phosphate Dehydrogenase (cytoplasmic)
- Glutamate Dehydrogenase
- Glyceraldehyde 3 phosphate Dehydrogenase.

NADP + utilizing enzymes

Mainly for Reductive Biosynthesis[Q] of steroids and Cholesterol[Q], Free radical Scavenging[Q], Formation of deoxyribonucleotides, One carbon metabolism.

- 3 Keto acyl reductase
- Enoyl reductase
- HMG CoA Reductase
- Folatereductase
- Glutathione Reductase
- Ribonucleotide Reductase.

Contd...

Contd...

NADPH generating Reactions[Q]

- Glucose 6 Phosphate Dehydrogenase in HMP shunt pathway
- 6 PhosphoGluconate Dehydrogenase in HMP shunt pathway
- Cytoplasmic Isocitrate Dehydrogenase
- Malic Enzyme. (NADP Malate Dehydrogenase).

Other function of NAD

*NAD is the **source of ADP-ribose** for the ADP-ribosylation of proteins and polyADP-ribosylation of nucleoproteins involved in the DNA repair mechanism.*

Deficiency of niacin

Pellagra

- Photosensitive Dermatitis: Symmetric dermatitis in the sun exposed areas
- Skin lesions are dark, dry and scaling
- Casal's Necklace[Q] The rash form a ring around the neck
- Dementia
- Insomnia, irritability, and apathy and progresses to confusion, memory loss, hallucination, and depressive psychosis
- Diarhea can be severe resulting in malabsorption due to atrophy of intestinal villi
- Advanced Pellagra can result in death
- Depressive psychosis.

4 Ds of Pellagra

- Dermatitis (Photosensitive Dermatitis)
- Dementia
- Diarrhea
- Death.

Conditions associated with Pellagra like symptoms

- Hartnup Disease (Due to intestinal malabsorption and renal reabsorption of Tryptophan)
- Carcinoid Syndrome (Over production of serotonin leads to diversion of Tryptophan from NAD+ pathway)
- Vitamin B6 deficiency (Defective Kynureninase that lead to defective synthesis of Niacin)
- Pellagra is common in people whose staple diet is maize and jowar.

Maize-Niacin present in unavailable form Niacytin

Sorghum vulgare (Jowar)-High Leucine content inhibit QPRTase, rate limiting enzyme in Niacin synthesis.

Recommended Daily Allowance of Niacin (RDA)

20 mg/day

Toxicity of niacin

- Prostaglandin mediated cutaneous flushing due to binding of vitamin to a G Protein coupled receptor
- Gastric irritation

- Hepatic toxicity is the most serious toxic reaction with sustained release niacin presents withjaundice, elevated liver enzymes (AST and ALT) even fulminant hepatitis
- Other toxic reactions include glucose intolerance, hyperuricemia, macular edema and cysts.

Treatment of cutaneous flushing

- Laropiprant, a selective Prostaglandin D2 receptor 1 antagonist
- Premedication with Aspirin.

Therapeutic uses of Niacin (Nicotinic acid)

- Used as Lipid modifying Drug
- Niacin reduces plasma triglyceride and LDL-C levels and raises the plasma concentration of HDL-C.

Pyridoxine (Vitamin B6)

Family of 3 related Pyridine derivatives

- Pyridoxine
- Pyridoxal
- Pyridoxamine

Remember

Some 80% of the body's total vitamin B6 is pyridoxal phosphate in muscle[Q], mostly associated with glycogen phosphorylase.

Active form of Pyridoxine

- Pyridoxal Phosphate (PLP)
- Mainly used for Amino Acid metabolism[Q].

Coenzyme Role of Pyridoxal Phosphate (PLP)[Q]

Transamination

- Alanine Amino Transferase (ALT)
- Aspartate Amino Transferase (AST)
- Alanine Glyoxalate Amino Transferase.

Decarboxylation of amino acids

This results in the formation of Biogenic Amines

- Glutamate: GABA
- 5-Hydroxy Tryptophan: Serotonin
- Histidine: Histamine
- Cysteine: Taurine
- Serine: Ethanolamine
- DOPA: Dopamine.

Transulfuration

- Involved in the metabolism of Sulfur containing amino acids
- Synthesis of Cysteine from methionine
- Enzymes are Cystathionine Beta Synthase and Cystathioninase.

Tryptophan metabolism

- Coenzyme of **Kynureninase** involved in the synthesis of niacin from Tryptophan
- In Pyridoxine deficiency **Xanthurenic acid** is excreted because of defective Kyneureninase in Niacin synthesis.

Heme synthesis

- ALA Synthase that catalyse condensation of Succinyl CoA and Glycine.

Glycogenolysis

- Glycogen phosphorylase.

Deficiency of Vitamin B6 (Pyridoxine)

- Neurological manifestation: Due to deficiency of Catecholamines
- Peripheral neuropathy
- Personality changes that include depression and confusion
- Convulsions: Due to decreased synthesis of GABA
- **Microcytic hypochromic Anemia**: Due to decreased heme synthesis
- Pellagra due defective niacin synthesis.

Other conditions caused by PLP deficiency.

- *Oxaluria:* Due to defective Alanine: Glyoxylate Amino Transferase. Glyoxylate converted to Oxalic acid
- *Homocystinuria:* Due to defective Cystathionine Beta Synthase
- *Xanthurenic Aciduria:* Due to defective Kynureninase
- *Cardiovascular risks:* Because of homocysteinemia.

Drugs that interact with carbonyl group and causes PLP deficiencies are L-Dopa, Pencillamine, Cycloserine.

Pyridoxine dependency syndromes that need pharmacological dose of PLP

- Classic homocystinuria (due to cystathionine beta synthase deficiency)
- Sideroblastic anemia (due to ALA Synthase deficiency)
- Gyrate atrophy of retina and choroid in δ- ornthine amino transferase.

High doses of Pyridoxine given in

- Carpal Tunnel syndrome
- Premenstrual syndrome
- Schizophrenia
- Diabetic neuropathy.

Pyridoxine and Hormone dependent cancer

- Pyridoxine is important in steroid hormone action

Contd...

Contd...

- Pyridoxal phosphate removes the hormone-receptor complex from DNA binding, terminating the action of the hormones
- In vitamin B6 deficiency, there is increased sensitivity to the actions of low concentrations of estrogens, androgens, cortisol, and vitamin D
- Increased sensitivity to steroid hormone action may be important in the development of hormone-dependent cancer of the breast, uterus, and prostate, and vitamin B6 status may affect the prognosis.

Biochemical Assay of Vitamin B6

- Erythrocyte Transaminase activity
- Tryptophan load test-measurement of Xanthurenic acid following Tryptophan load
- Measurement of PLP in the blood.

Toxicity of Vitamin B6

- Excess Pyridoxine may lead to *Sensory Neuropathy*.

RDA of Pyridoxine

- 1–2 mg/day
- RDA of Pyridoxine depends on Protein intake.

Pantothenic Acid (Vitamin B5)

- Derived from the Greek word pantos means everywhere
- Endogenously synthesized by bacterial flora in the intestine
- Vitamin that contains Beta Alanine
- Vitamin present in Coenzyme A (CoA) and Acyl Carrier Protein (ACP) in Fatty Acid Synthase Complex.

The important CoA Derivatives are

- Acetyl CoA
- Succinyl CoA
- HMG CoA
- Acyl CoA.

Pantothenic acid as a part of CoA take part in

- Fatty acid Oxidation
- Acetylation
- Citric acid cycle
- Cholesterol synthesis.

Deficiency of Pantothenic Acid

- Gopalan's Burning feet Syndrome or **Nutritional Melalgia** or Peripheral nerve damage.

RDA of Pantothenic acid

10 mg/day.

Pantothenate kinase associated neurodegeneration (PKAN) (formerly Hallervorden-Spatz syndrome)

- Rare autosomal recessive neurodegenerative disorder
- Chorea, dystonia, parkinsonian features, pyramidal tract features and MR
- MRI-decreased T2 signal in the globuspallidus and substantianigra, 'eye of the tiger' sign (hyperintense area within the hypointense area)
- Sometimes acanthocytosis
- Neuropathologic examination indicates excessive accumulation of iron-containing pigments in the globuspallidus and substantianigra
- Similar disorders are grouped as neurodegeneration with brain iron accumulation (NBIA).

Biotin or Vitamin H or Vitamin B7

- Also known as anti-egg white injury factor
- Endogenously synthesized by intestinal flora
- Reactive form is the enzyme bound **CarboxyBiocytin.**

Coenzyme role of Biotin

Play a role in gene expression, fatty acid synthesis, gluconeogenesis and serve as a CO_2 carrier for Carboxylases enzymes and gene regulation by histone biotinylation.

Coenzyme for ATP dependent Carboxylation reaction (Carbon Dioxide Fixation)

- Pyruvate Carboxylase (Pyruvate to Oxaloacetate)
- Propionyl CoA Carboxylase (Propionyl CoA to Methyl Malonyl CoA)
- Acetyl CoA Carboxylase (Acetyl CoA to Malonyl CoA)
- Methyl Crotonyl CoA Carboxylase.

Biotin independent Carboxylation reaction

- Carbamoyl Phosphate Synthetase –I and II
- Addition of CO_2 to C6 in Purine ring (AIR Carboxylase)
- Malic Enzyme (Pyruvate to Malate).

- Gamma Carboxylation (Vitamin K dependent).

Biotin Antagonist

Avidin

- Protein present in the raw egg white
- Eating raw egg is harmful because of Avidin present in raw egg inhibit biotin
- Affinity of Avidin to Biotin is stronger than most of the Antigen antibody reaction.

This property is used in

- ELISA test
- Labelling of DNA.

Streptavidin

- Purified from Streptomyces avidinii
- Bind 4 molecules of Biotin.

Deficiency of biotin

- Mental changes (Depression, hallucination) paresthesia, anorexia, and nausea
- A scaling, seborrheic and erythematous rash around nose, eyes and mouth.

Biochemical tests to diagnose Biotin deficiency

- Decreased concentration of Urinary biotin
- Increased urinary excretion of 3-hydroxyvaleric acid after leucine challenge
- Decreased activity of biotin dependent enzymes in lymphocytes.

Folic Acid or Vitamin B9

- Derived from latin word folium, which means leaf of vegetable
- Folic Acid is abundant in leafy vegetables
- Folic Acid is absorbed from upper part of Jejunum[Q].

Functions of folic acid

- Active form of Folic acid is **Tetra Hydro Folic Acid (THFA)**
- THFA is the carrier of One Carbon groups.

One carbon metabolism

One carbon units are:

- Methyl (CH_3)
- Methylene (CH_2)
- Methenyl (CH)
- Formyl (CHO)
- Formimino (CH = NH).

One carbon groups bind to THF through

- N^5 are Formyl, Formimino or methyl
- N^{10} are Formyl
- Both N^5 and N^{10} are Methylene and Methenyl.

Sources of one carbon groups

- The major point of entry of one carbon unit is Methylene THF[Q]
- Serine[Q] is the most important source of One Carbon units
- Serine[Q]Hydroxy Methyl Transferase[Q] is the enzyme involved in this pathway.

Important sources of one carbon groups

Source of Methylene THF

- Serine to Glycine by Serine Hydroxy Methyl Transferase
- Glycine
- Choline.

Source of Formimino THF

- Histidine ---->FIGLU------->Formimino THF

Utilization of one carbon groups

- Serine to Glycine
- Homocysteine to Methionine
- Synthesis of Purine Nucleotides
- Synthesis of TMP
- Synthesis of Choline.

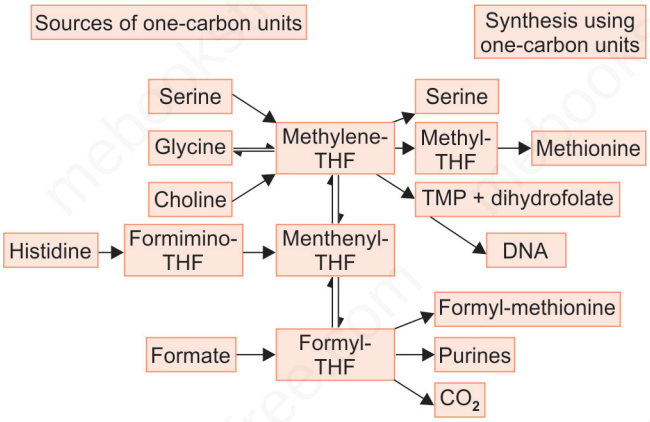


Fig. 15.3: One carbon metabolism

Pharmaceutically used THFA derivative

- ***5-Formyl-tetrahydrofolate[Q2013] is more stable than folate and is therefore used pharmaceutically (known as folinic acid),*** and the synthetic (racemic) compound **(leucovorin).**
- It is given orally or parenterally to overcome the toxic effects of methotrexate or other DHF reductase inhibitors.

Biochemical assessment of folate deficiency

- Serum Folate (Normal level is 2–20 ng/ml)
- Red Cell Folate
- Histidine Load test[Q] or FIGLU excretion test
- AICAR [Amino Imidazole Carboxamide Ribose 5 Phosphate] Excretion Test
- Serum Homocysteine
- Peripheral Blood Smear (Macrocytes, tear drop cells, hypersegmented neutrophils, anisopoikilocytosis).

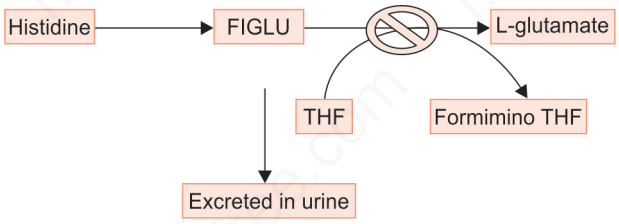


Fig. 15.4: FIGLU excretion

Deficiency of folic acid

- **Reduced DNA Synthesis** because THF derivatives are involved in purine synthesis and thymidylate Synthesis
- **Megaloblastic Anemia**
  - Vitamin B12 deficiency and Folate deficiency can lead to this condition
  - In Vitamin B12 deficiency Megaloblasticanemia is due to folate trap
- **Homocysteinemia** due decreased conversion of Homocysteine to Methionine. This is because Methyl THFA is the methyl donor for this reaction
- **Neural tube defects (like Spina bifida)** during pregnancy
- Atrophic glossitis
- Depression.

Folic acid and cancer

- Low folate status results in impaired methylation of CpG islands in DNA, which is a factor in the development of **colorectal and other cancers**
- Prophylactic Folic Acid during pregnancy reduce chance of **Acute Lymphoblastic Lymphoma**
- But, folate supplements increase the rate of transformation of preneoplastic colorectal polyps into cancers
- Folic acid 'feed' tumors by increasing thymidine pools and 'better' quality DNA
- So Folic Acid should be avoided in established tumors.

Vitamin B12 (Cobalamin)

- Other name is Extrinsic factor of castle
- Contain **4.35% cobalt by weight**
- Contain 4 pyrrole rings coordinated with a cobalt atom, called Corrin ring.

Active forms of Vitamin B12

- Methyl Cobalamin and Adenosyl Cobalamin (Ado B12)

Coenzyme Role of Cobalamin

- Methyl Malonyl CoA Mutase
- L Methyl Malonyl CoA → Succinyl CoA Methionine Synthase or Homocysteine Methyl Transferase
- Homocysteine → Methionine
- Leucine Amino Mutase

Vitamin B12 metabolism

Absorption of cobalamin

- 99% of absorption of Cobalamin are active
- Active mechanism: Site is Ileum[Q]
- 1% passive occurs equally in Buccal cavity, Duodenum, Ileum.

Cobalamin binding proteins

- Cobalamin binding proteins in the saliva are called Haptocorrins or Cobalophilin or R Binders
- Intrinsic Factor of Castle from parietal cells of body and fundus of the stomach
  - Vitamin B12 is freed from binding proteins in food through the action of pepsin in the stomach and binds to salivary proteins called **cobalophilins, or R-binders**
  - In the duodenum, bound vitamin B12 is released by the action of pancreatic proteases. It then associates with intrinsic factor
  - Actively absorbed from the **ileum**[Q] by binding to IF receptor
  - IF receptor in the ileum is called **CUBULIN.**

Transport of Cobalamin to the target tissues

- Major Cobalamin transport protein in plasma is Transcobalamin II (TC II)[Q]
- Transcobalamin I [TC I] play a role in the transport of Cobalamin analogues
- At the target tissues by receptor mediated endocytosis involving TC II receptor.

Causes of Vitamin B12 deficiency

Nutritional

- Vitamin B12 is found only in foods of animal origin, there being no plant sources of this vitamin. *This means that strict vegetarians (vegans) are at risk of developing B12 deficiency.*

Malabsorption-pernicious anemia

- Pernicious anemia is a specific form of megaloblasticanemia caused by autoimmune gastritis and an attendant failure of intrinsic factor production, which leads to vitamin B12 deficiency.

Gastric causes

- Congenital absence of intrinsic factor or functional abnormality
- Total or partial gastrectomy.

Intestinal causes

- Intestinal stagnant loop syndrome: jejunal diverticulosis, ileocolic fistula, anatomic blind loop, intestinal stricture, etc.
- Ileal resection and Crohn's disease.

Selective malabsorption with proteinuria

- Imerslund Syndrome

- Imerslund-Gräsbeck Syndrome
- Congenital Cobalamin Malabsorption
- Autosomal Recessive Megaloblastic Anemia
- Tropical sprue
- Transcobalamin II deficiency.

Fish tapeworm

- The fish tapeworm (*Diphyllobothriumlatum*) lives in the small intestine of humans and accumulates cobalamin from food, rendering the cobalamin unavailable for absorption.

Vitamin B12 deficiency and Folate trap

- When acting as a methyl donor, *S*-adenosyl methionine forms homocysteine, which may be remethylated by methyl-tetrahydrofolate catalyzed by methionine synthase, a vitamin B12–dependent enzyme
- The reduction of methylene-tetrahydrofolate to methyl-tetrahydrofolate is irreversible. This is the major source of tetrahydrofolate for tissues is methyltetrahydrofolate
- Impairment of methionine synthase in vitamin B12 deficiency results in the accumulation of methyltetrahydrofolate—the 'folate trap'
- There is therefore functional deficiency of folate, secondary to the deficiency of vitamin B12.

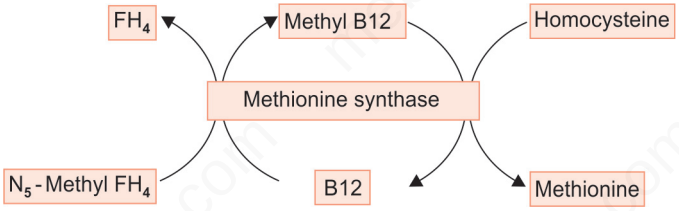


Fig. 15.5: Folate trap

Deficiency manifestation of Vitamin B12

- Megaloblasticanemia
- **Homocysteinemia**: Due decreased conversion of Homocysteine to Methionine
- **Methyl Malonic Aciduria**: Due to defective Methyl Malonyl CoA Mutase which leads to decreased conversion of L Methyl malonyl CoA to Succinyl CoA
- **Subacute Combined Degeneration**
- Cobalamin deficiency may cause a bilateral peripheral neuropathy or degeneration (demyelination) of the posterior and pyramidal tracts of the spinal cord.

Biochemical assessment of cobalamin deficiency

- Serum Cobalamin
- Serum Methyl Malonate (This helps to distinguish between Megaloblasticanemia due to Cobalamin deficiency and Folate deficiency)
- Serum Homocysteine
- Schilling Test using Radioactive labelled Cobalt-60
- Urine Homocystine and MMA
- Bone marrow and Peripheral Blood Smear.

Vitamin C (Ascorbic Acid)

- Other name is antiscorbutic factor
- Most animals synthesize Vitamin C from Glucose by uronic Acid Pathway[Q]
- Humans and higher Primates cannot due to absence of Gulonolactone Oxidase[Q].

Biochemical Functions of Ascorbic Acid

- Acts as a good reducing agent and a scavenger of free radicals (Antioxidant)
- In Collagen Synthesis: Vitamin C is required for the post-translational modification, Hydroxylation of lysine and Proline
- Hydroxylation of Tryptophan
- Tyrosine Metabolism: Oxidation of P hydroxyl Phenyl Pyruvate to Homogentisic Acid
- Bile Acid Synthesis in 7 alpha Hydroxylase
- Iron Absorption: Favor Iron absorption by conversion of Ferric ions to Ferrous ions
- Folate Metabolism: Conversion of Folate to its active form
- Adrenal steroid synthesis.

Vitamin C Deficiency

Scurvy

- Petechiae, ecchymosis, coiled hairs, inflamed and bleeding gums, joint effusion, poor wound healing, fatigue
- Perifollicular hemorrhages
- Perifollicular hyperkeratotic papules, petechiae, purpura
- Splinter hemorrhage, bleeding gums, hemarthroses, subperiosteal hemorrhage
- Anemia
- Late stage are characterized by edema, oliguria, neuropathy, intracerebral hemorrhage and death.

Barlows Syndrome (Infantile Scurvy)

- In infants between 6-12 months, the diet if not supplemented with Vitamin C then deficiency will result.

Vitamin C toxicity

- Gastric irritation, flatulence, diarrhea,
- Oxalate stones are of theoretic concern.

Vitamins at a Glance

Vitamin deficiencies causing dementia

- Thiamin
- Niacin
- Cobalamin

Sulfur Containing Vitamins

- Biotin
- Thiamin

Antioxidant Vitamin

- Vitamin E
- Vitamin C
- Beta Carotene

Antioxidant vitamins are also Pro-oxidants

- Vitamin C
- Beta Carotene
- Vitamin E

B complex Vitamins with Toxicity

- Niacin
- Pyridoxine

Redox Vitamins

Vitamins that take part in Oxidation reduction reaction

- Niacin and Riboflavin

Endogenously Synthesized Vitamins

- Niacin (Vitamin B3)
- Biotin
- Vitamin D
- Pantothenic Acid
- Vitamin K.

Ring Structures of B-complex Vitamins

Vitamin	Ring structure
Vitamin B1 [Thiamine]	Pyrimidine + Thiazole
Vitamin B2 [Riboflavin]	Isoalloxazine
Vitamin B3 [Niacin]	Pyridine
Vitamin B6 [Pyridoxine]	Pyridine
Vitamin B12 [Cobalamin]	Corrin [Tetrapyrrole with Co at its center]
Folic Acid	Pteridine + PABA
Biotin	Imidazole + Thiophene
Pantothenic Acid	**No ring Structure** Contain Pantoic Acid and **Beta Alanine**[Q] in amide linkage

Deficiency of Vitamins

Principal clinical findings of vitamin malnutrition	
Nutrient	**Clinical finding**
Thiamin	**Peripheral nerve damage** (beriberi) or central nervous system lesions (Wernicke-Korsakoff syndrome)
Riboflavin	Magenta tongue, angular stomatitis, cheilosis, **seborrheic dermatitis**
Niacin	Pellagra: pigmented rash of sun-exposed areas (photosensitive dermatitis), bright red tongue, diarrhea, apathy, memory loss, disorientation, depressive psychosis
Vitamin B6	Seborrhea, glossitis, convulsions, neuropathy, depression, confusion, microcytic anemia
Folate	Megaloblasticanemia, atrophic glossitis, depression, ↑homocysteine
Vitamin B12	Pernicious anemia = megaloblasticanemia with **degeneration of the spinal cord**, loss of vibratory and position sense, abnormal gait, **dementia**[Q], impotence, loss of bladder and bowel control, ↑**homocysteine**, ↑**methylmalonic acid**
Pantothenic Acid	Peripheral nerve damage (nutritional melalgia or 'burning foot syndrome')
Vitamin C	Scurvy: petechiae, ecchymosis, coiled hairs, inflamed and bleeding gums, joint effusion, poor wound healing, fatigue
Vitamin A	Xerophthalmia, night blindness, Bitot's spots, follicular hyperkeratosis, impaired embryonic development, immune dysfunction
Vitamin D	Rickets: skeletal deformation, rachitic rosary, bowed legs; osteomalacia
Vitamin E	Peripheral neuropathy, spinocerebellar ataxia, skeletal muscle atrophy, retinopathy
Vitamin K	Elevated prothrombin time, bleeding

MINERALS

Classified into

- Macrominerals (Major elements)
  - Daily requirement > 100 mg
  - Calcium, Magnesium, Phosphorus, Sodium, Potassium, Chloride, Sulfur
- Micromineral (Trace element)
  - Daily requirement < 100 mg
  - Iron, Iodine, Copper, Cobalt, Mangenese, Molybdenum, Selenium, Zinc, and Fluorine
- Ultra trace elements
  - Daily requirement < 1 mg/day.

IRON

Body distribution of Iron.

	Iron content, mg	
	Adult male	Adult female
Hemoglobin	2500	1700
Myoglobin/Enzymes	500	300
Transferrin	3	3
Iron Stores	600–1000	0–300
Total Body Iron content	**3603–4003**	**2003–2303**

Iron Containing Proteins

Heme Containing[Q]
- Hemoglobin
- Myoglobin
- Cytochrome c
- Cytochrome oxidase
- Tryptophan pyrrolase
- Catalase
- Nitric Oxide Synthase

Nonheme –iron containing Proteins
- Aconitase
- Transferrin
- Ferritin
- Hemosiderin

Iron-Sulfur Complex
- Complex I of ETC
- Complex II of ETC
- Complex III of ETC
- Xanthine oxidase

Proteins that has role in Iron metabolism

Storage form Ferritin and Hemosiderin

Ferritin

- The human body can typically store up to 1 g of iron, the vast majority of which is bound to **ferritin**
- MW 440 kDa
- Ferric iron + Apoferritin = Ferritin
- Poly nuclear complex of hydrous ferric oxide
- Ferritin is composed of 24 identical subunits, which surround as many as 3000 to 4500 ferric atoms
- The subunits may be of the H (heavy) or the L (light) type
- The H-subunit possesses ferroxidase activity, which is required for iron-loading of ferritin
- The function of the L subunit is not clearly known but is proposed to play a role in ferritin nucleation and stability
- Seen in Intestinal cells, Liver, Spleen and Bone marrow
- Plasma ferritin levels thus are considered to be an **indicator of body iron stores.**

Hemosiderin

- A partly degraded form of ferritin that contains iron is Hemosiderin
- Iron is not easily mobilized from Hemosiderin unlike ferritin
- It can be detected in tissues by histological stains (e.g. Prussian blue), under conditions of iron overload (**hemosiderosis**)
- Hemosiderin is an Index of Iron Overload[Q].

Transport form Transferrin

Transferrin and Transferrin receptors

- Iron is transported in plasma in the **Fe^{3+}** form by the transport protein, **transferrin**
- Ferric iron combines with apo transferrin to form transferrin
- Synthesized in the **Liver**
- Transferrin is a **β1 globulin**
- Transferrin is a **bilobed glycoprotein** with two iron binding sites
- Transferrin that carries iron exists in two forms—***monoferric*** **(one iron atom) or** ***diferric*** **(two iron atoms)**
- The turnover (half-clearance time) of transferrin-bound iron is very rapid—typically **60–90 min**
- Normal **1/3rd transferrin** saturated with Iron
- The iron-transferrin complex circulates in the plasma until it interacts with specific *transferrin receptors*
- On the surface of marrow erythroid cells
- **Diferric transferrin** has the highest affinity for transferrin receptors
- The greatest number of transferrin receptors (300,000 to 400,000/cell) is the **developing erythroblast**
- **The Transferrin receptor 1 (TfR1**) can be found on the surface of most cells
- **Transferrin receptor 2 (TfR2)**, by contrast, is expressed primarily on the surface of hepatocytes and also in the crypt cells of the small intestine
- The affinity of TfR1 for Tf-Fe is much higher than that of TfR2
- The major role of TfR2 is sensing iron level, rather than internalizing iron.

Reciprocal regulation of TfR1 and Ferritin
- The rates of synthesis of TfR1 and ferritin are reciprocally linked to intracellular iron levels
- When iron is low, TfR1 synthesis increases and that of ferritin declines

Contd...

Contd...

- The opposite occurs when iron is abundant
- Control is exerted through the binding of iron regulatory proteins (IRPs) called **iron response elements (IREs)** located in the 5' and 3' untranslated regions of mRNA.

Concept

When iron level is low, tissue demand for iron is high, increased transferrin receptors, help to internalize the available iron in the plasma. Decreased ferritin will help to mobilize the maximum iron stores to meet the demand of iron.

Carbohydrate Deficient Transferrin (CDT)

- Glycosylation of transferrin is impaired in **congenital disorders of glycosylation** as well as in **chronic**
- Alcoholism
- The presence of **carbohydrate-deficient transferring (CDT)**, which can be measured by isoelectric focussing (IEF)
- This is used as a biomarker of chronic alcoholism and Congenital Disorders of Glycosylation (CDGs)

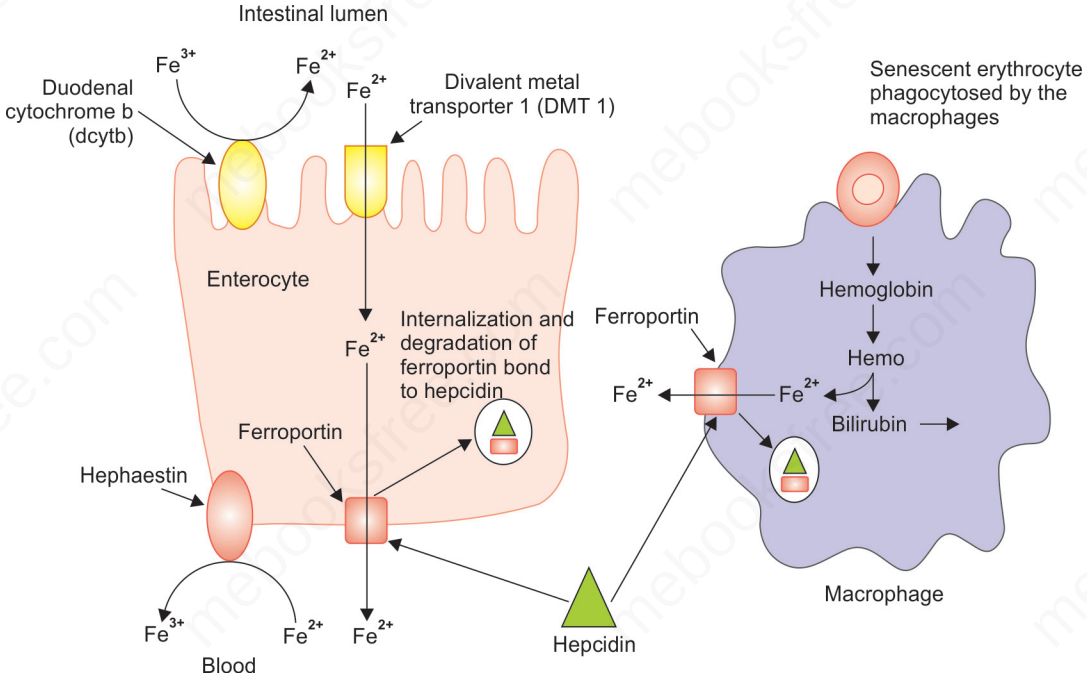


Fig. 15.6: Metabolism of iron

Iron Metabolism

- Site of absorption: Enterocytes in the **proximal duodenum**
- Heme iron is absorbed by a **heme transporter**
- Iron is absorbed in the **ferrous** form[Q]
- **Inorganic dietary iron** in the ferric state (Fe^{3+}) is reduced to its ferrous form (Fe^{2+}) by a brush border membrane-bound ferrireductase, **duodenal cytochrome b (Dcytb)**
- **Vitamin C** in food also favors reduction of ferric iron to ferrous iron
- The transfer of iron from the apical surfaces of enterocytes into their interiors is performed by a proton- coupled **divalent metal transporter (DMT1)**
- This protein is not specific for iron, as it can transport a wide variety of divalent cations. (Co^{2+},Zn^{2+},Pb^{2+},Cu^{2+})

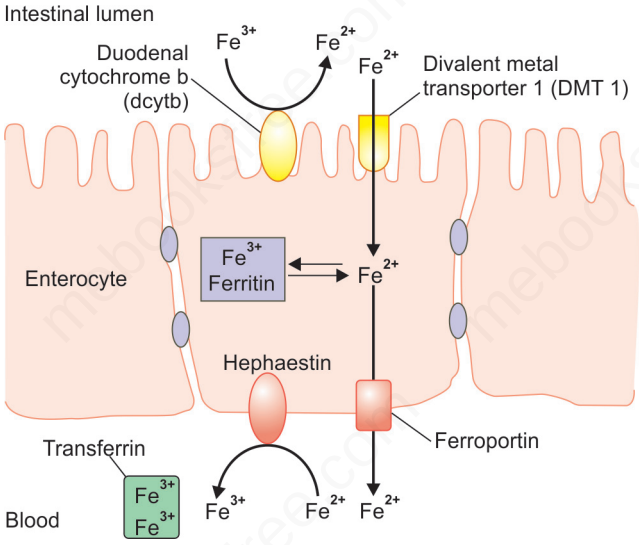


Fig. 15.7: Absorption of Iron

- Once inside the enterocytes, iron can either be stored as **ferritin** or transferred across the basolateral membrane into the circulation by the iron exporter protein, **ferroportin** or **iron-regulated protein 1 (IREG1** or **SLC40A1)**
- This protein may interact with the copper-containing protein **hephaestin,** a protein similar to ceruloplasmin
- Hephaestin is thought to have a ferroxidase activity, which is important in the release of iron from cells
- Thus, Fe^{2+} is converted back to Fe^{3+}, the form in which it is transported in the plasma by transferrin.

Dietary Regulation of Iron by Mucosal Block at the Level of Enterocyte

Hepcidin

- Hepcidin is the Chief Regulator of Systemic Iron Homeostasis
- It is a 25-amino acid peptide
- Synthesized in the liver as an 84-amino acid precursor (prohepcidin).

Mechanism of Iron regulation by hepcidin

- Hepcidin binds to the cellular iron exporter, ferroportin, triggering its internalization and degradation
- The consequent decrease in ferroportin results in decreased export of iron into circulation and depressed iron recycling by macrophages
- Together, these result in a reduction in circulating iron levels (hypoferremia) as well as reduced placental iron transfer during pregnancy
- When plasma iron levels are high, hepatic synthesis of hepcidin increases, thus reducing circulating iron level
- The opposite occurs when plasma iron levels are low.

Regulation of expression of hepcidin

The hepcidin level is influenced by

- Circulatory level of iron
- Bone Morphogenic Proteins (BMPs) and Hemojuvelin
- Erythropoietic signals
- Inflammation
- Hypoxia

Circulation level of Iron

- Liver cells monitor iron levels using an iron sensing complex comprised of two transmembrane receptors (TfR-1, TfR-2) and transmembrane protein HFE protein
- TfR-1 binds to iron bound transferring (Tf-Fe) at the site where it binds to HFE protein
- When iron is abundant, Tf-Fe are high, hence HFE is displaced from TfR-1
- The displaced HFE binds to TfR-2
- Binding of HFE to TfR-2 triggers intracellular signal cascade (ERK-MAPK cascade)
- Which activate expression of HAMP gene that codes for Hepcidin.

Concept is increased level of iron→increased expression of hepcidin→which inturn decreases circulating iron.

Bone Morphogenic Proteins (BMPs) and Hemojuvelin (HJV)

- BMP binds to a cell-surface receptor (BMPR) whose binding affinity is augmented by binding to a co-receptor, hemojuvelin (HJV)
- The activation of the BMPR-HJV complex triggers the phosphorylation of intracellular signaling proteins called SMADs, which subsequently results in **transcriptional activation of hepcidin.**

Erythropoietic signals

- Two molecules secreted by erythroblasts, growth differentiation factor 15 (GDF15) and twisted gastrulation 1 (TWSG1)
- They **inhibit expression of hepcidin** in β-thalassemia major.

Inflammation

- **Hepcidin synthesis is induced** by cytokines such as interleukin–6 (IL-6) that are released as part of an inflammatory response
- Binding of IL-6 to its cell surface receptor stimulates gene expression by activating the JAK-STAT (Janus Kinase—Signal Transducer and Activator of Transcription) Pathway
- Anemia that is associated with chronic inflammation (anemia of inflammation or AI) is probably due to inflammation-mediated upregulation of hepcidin.

Hypoxia

- Hypoxia is **suppress hepcidin expression**
- This effect is mediated by erythropoietin, whose synthesis is controlled by hypoxia-inducible transcription factors 1 and 2 (HIF-1 and HIF-2).

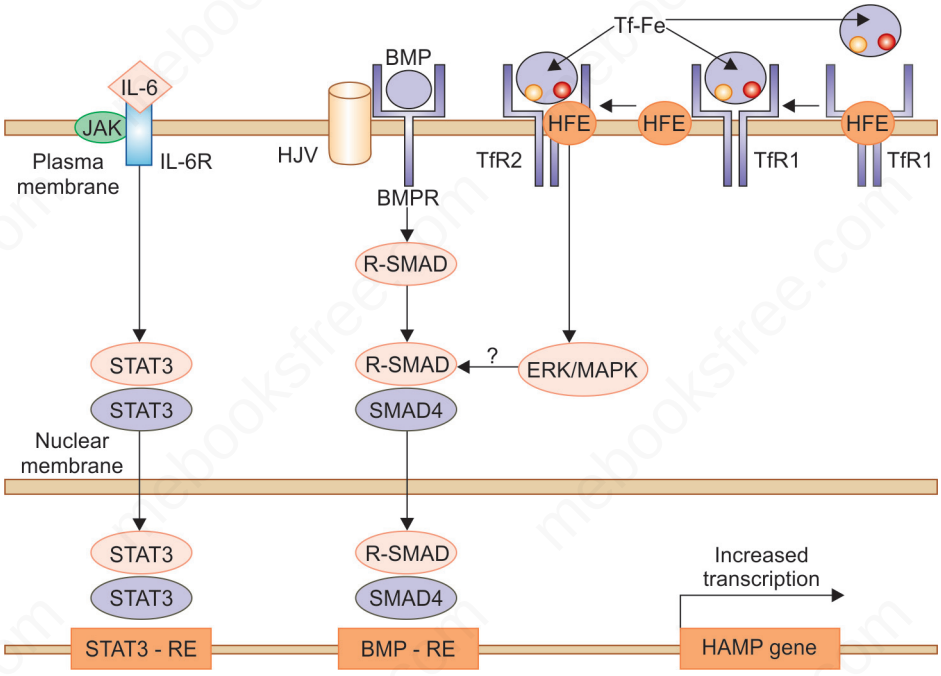


Fig. 15.8: Regulation of Expression of Hepcidin

Conservation of Iron

- Extracorpuscular hemoglobin is bound by **haptoglobin**
- **Hemopexin** is a β1 globin that binds Heme
- **Albumin** will bind some metheme (ferric heme) to form methemalbumin
- Which then transfers the metheme to hemopexin
- Transferrin bind **free Iron (Fe $^{3+}$)** in plasma.

Haptoglobin

- Human haptoglobin exists in **three polymorphic forms,** known as Hp 1-1, Hp 2-1, and Hp 2-2
- Haptoglobin is an acute phase protein, and its plasma level is elevated in a variety of inflammatory states
- Haptoglobin scavenges hemoglobin that has escaped recycling.

Haptoglobin protects the kidneys from damage by extracorpuscular hemoglobin

- During the course of red blood cell turnover, approximately 10% of an erythrocytes hemoglobin is released into the circulation.
- This free, **extracorpuscular** hemoglobin is sufficiently small at =65 kDa to pass through the glomerulus of the kidney into the tubules, where it tends to form damaging precipitates.
- **Haptoglobin** (Hp) is a plasma glycoprotein that binds extracorpuscular hemoglobin (Hb) to form a tight noncovalent complex (Hb-Hp).
- Since the Hb-Hp complex is too large (≥155 kDa) to pass through the glomerulus, this protects the kidney from the formation of harmful precipitates and reduces the loss of the iron associated with extracorpuscular hemoglobin.

Contd...

Contd...

Haptoglobin level in hemolytic anemia

- Patients suffering from **hemolytic anemias** exhibit low levels of haptoglobin
- The half-life of haptoglobin is approximately 5 days
- The Hb-Hp complex is removed rapidly by the hepatocytes (half-life 90 minutes)
- Thus, when haptoglobin is bound to hemoglobin, it is cleared from the plasma about 80 times faster than normally
- So the level of haptoglobin falls rapidly in situations where hemoglobin is constantly being released from red blood cells, such as occurs in hemolytic anemias.

Haptoglobin-related protein and cancer

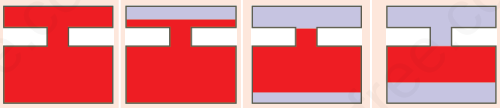
A plasma protein that has a high degree of homology to haptoglobin, it is elevated in some patients with cancers, although the significance of this is not understood.

Iron Deficiency Anemia

Stages of iron deficiency

- The progression to iron deficiency can be divided into three stages
- The first stage is **negative iron balance**, in which the demands for (or losses of) iron exceed the body's ability to absorb iron from the diet. Serum iron is normal and hemoglobin synthesis is unaffected
- The second stage is ***iron-deficient erythropoiesis,*** transferrin saturation falls to 15–20%, Serum iron level begin to fall, hemoglobin synthesis becomes impaired

- The third stage is **Iron deficiency anemia**, where hemoglobin and hematocrit falls. Microcytic Hypochromic anemia sets in.

	Normal	Negative iron balance	Iron deficient erythro-poiesis	Iron de-ficiency anemia
Iron stores Erythron iron				
Marrow iron stores	1–3 +	0–1 +	0	0
Serum ferritin (µg/L)	50–200	< 20	< 15	< 15
TIBC (µg/dL)	300–360	> 360	> 380	> 400
SI (µg/dL)	50–150	NL	< 50	< 30
Saturation (%)	30–50	NL	< 20	< 10
Marrow sideroblasts (%)	40–60	NL	< 10	< 10
Protopor-phyrin (µg/dL)	30–50	NL	> 100	> 200
RBC morphology	NL	NL	NL	Microcytic/ hypochronic

Laboratory iron studies in normal and different stages of evolution of iron deficiency

Parameter	Normal	Negative iron balance	Iron defi-cient eryth-ropoiesis	Iron deficiency anemia
Marrow Iron stores	1–3+	0–1 +	0	0
Serum ferritin (µg/dL)	50–200	Decreased < 20	Decreased < 15	Decreased < 15
Total iron bind-ing capacity (TIBC) (µg/dL)	300–360	Slightly increased > 360	Increased > 380	Increased > 400
Serum iron (µg/dL)	50–150	Normal	Decreased < 50	Decreased < 30
Transferrin saturation (%)	30–50	Normal	Decreased < 20	Decreased < 10
RBC protopor-phyrin (µg/dL)	30–50	Normal	Increased	Increased
Soluble trans-ferrin receptor (µg/L)	4–9	Increased	Increased	Increased
RBC morphol-ogy	Normal	Normal	Normal	Microcytic Hypochro-mic

Lab Parameters that increase in Iron Deficiency Anemia

- TIBC
- RBC Protoporphyrin
- s TR[TRP](Transferrin Receptor Protein)
- RBC Distribution Width[RDW]

Diagnosing Microcytic anemia				
Tests	**Iron defi-ciency**	**Inflamma-tion**	**Thalas-semia**	**Sidero-blastic anemia**
Peripheral Smear	Microcytic Hypochro-mic	Normal/ Micro/ Hypo	Microcytic Hypochro-mic with targeting	Variable
S Iron (µg/dL)	< 30	< 50	Normal to high	Normal to high
TIBC(µg/dL)	> 360	**< 300**	**Normal**	**Normal**
Transferrin satura-tion(%)	< 10	10–20	30–80	30–80
Ferritin(µg/L)	**< 15**	30–200	50–300	50–300
Hb electro-phoresis pattern	Normal	Normal	**Abnormal pattern in beta Thal-assemia**	Normal

Iron Overload Conditions

TYPE-I Hereditary Hemochromatosis (HFE related)

- Mutation in HFE gene located on Chr 6p
- Tightly linked to the HLA-A locus
- Most Common Hemochromatosis [80–90%]

Non HFE related Hereditary Hemochromatosis

- Juvenile hemochromatosis (type 2A) (hemojuvelin mutations)
- Juvenile hemochromatosis (type 2B) (hepcidin mutation)
- Mutated transferrin receptor 2 *TFR2* (type 3)
- Mutated ferroportin 1 gene, *SLC11A3* (type 4)

Secondary Hemochromatosis

- Anemia characterized by ineffective erythropoiesis (eg, thalassemia major)
- Repeated blood transfusions
- Parenteral iron therapy
- Dietary iron overload (Bantu siderosis)

Miscellaneous Conditions Associated with Iron Overload

- Alcoholic liver disease
- Nonalcoholic steatohepatitis
- Hepatitis C infection.

Hemochromatosis

Inherited disorder of iron metabolism that lead to iron overload, leading to deposition of iron in the parenchymal cells leading to fibrosis and organ failure.

Hemosiderosis

- **Acquired** condition
- Presence of stainable iron in tissues

Hemochromatosis at a glance

- The first organ to be affected in Hemochromatosis **Liver**
- Maximum deposition of Hemosiderin is seen in **Liver**
- Least Hemosiderin deposition is seen in **Skin**

Classical Triad of Hemochromatosis is

- Cirrhosis with Hepatomegaly
- Skin Pigmentation [Bronzing]

Due to the epidermis of the skin is thin, and melanin is increased in the cells of the basal layer and dermis

- Diabetes Mellitus
- First joint to be affected in hemochromatosis-2nd and 3rd MCP joint
- Most common cause of death in treated patients-Hepatocellular Carcinoma
- Role of HFE Mutations in other diseases
  - Nonalcoholic Steatohepatitis
  - Porphyria CutaneaTarda

COPPER

Cofactor role of Copper

- Amine oxidases
- Ferroxidase (ceruloplasmin) (Iron metabolism, Copper Transport)
- Cytochrome-*c* oxidase (in Complex IV of Electron Transport Chain)
- Superoxide dismutase (Free Radical Scavenging enzyme)
- Tyrosinase (Melanin Synthesis)
- Component of ferroportin (Iron Metabolism)
- Lysyl Oxidase (Cross linking in Collagen)

Copper Deficiency Anemia is a microcytic hypochromic type of Anemia.

Wilson's Disease

Autosomal recessive

Biochemical defect

- ATP7 B mutation, a gene encoding for Copper transporting ATPase in the cells
- Defective Biliary Copper Excretion from liver cells
- Defective Copper incorporation into Apoceruloplasmin
- Copper accumulate in cells leading to copper deposits in the liver and brain.

Quick glance: Wilson's disease

- The most common presentation in Wilson's disease: Acute or Chronic Liver Disease
- Neuropsychiatric manifestation in Wilson's resembles: Parkinson's Disease like Syndrome
- Most Sensitive test in Wilson's disease is or gold standard investigation is liver biopsy quantitative copper assay
- False positive is liver biopsy quantitative copper assay in obstructive liver disease
- The most specific Screening Test: Urinary Excretion of Copper

Diagnosis of Wilson's Disease

- 99% of cases Kayser-Fleischer ring is present, but absence of KF ring does not excludes the disease
- Serum Ceruloplasmin [18–35 mg/dL] decreased
- But normal in 10% of affected individuals and decreased in 20% of carriers
- 24 hour Urinary Copper > 100 µg/24 hr
- Gold Standard investigation^{Q} is Liver Biopsy^{Q} with quantitative Copper assays (> 200 µg/g dry weight of Liver)

Test	Usefulness	Normal Value	Wilson disease
Serum Ceruloplasmin	+	180–350 mg/L (18–35 mg/dL)	Low in 90%
Kayser-Fleischer ring	++	Absent	Present in > 99% if neurologic or psychiatric symptoms are present. Present 30–50% in hepatic presentation and presymptomatic patients
Urine Copper (24h)	+++	0.3–0.8 µmol (20–50 µg)	> 100 µg in symptomatic patients 60–100 µg in presymptomatic
Liver Copper	++++	0.3–0.8 µmol/g (20–50 µg/g of tissue)	> 3.1 µmol (> 200 µg)

Treatment

Disease status	First line	Second line
Hepatitis or Cirrhosis without decompensation	Zinc	Trientene
Hepatic decompensation		
Mild	Trientene and Zinc	Penicillamine and Zinc
Moderate	Trientene and Zinc	Hepatic Transplantation
Severe	Hepatic Transplantation	Trientene and Zinc
Initial neurologic/ psychiatric	Tetrathiomolybdate and Zinc	Zinc
Maintenance / Presymptomatic/ Pregnant/ Pediatric	Zinc	Trientene

Method to assess severity of Hepatic Decompensation in Wilson's disease

Nazer's Prognostic Index

- Serum Bilirubin
- Serum Aspartate Transferase [AST]
- Prolongation of Prothrombin Time
  - Score < 7 Medical management
  - Score > 9 Liver Transplantation

Menke's (Kinky or Steely) Hair Syndrome

- Mutation in *ATP7A gene*
- X linked recessive condition
- Defective Copper binding P-type ATPase
- Copper is not mobilized from **Intestine**

MEDNIK Syndrome

- A rare multisystem disorder of copper metabolism with features of both Wilson's and Menke's disease
- Caused by mutation in AP1S1 gene, which encodes an adaptor protein necessary for intracellular trafficking of ATP7 A and ATP7B.
- MEDNIK stands for Mental retardation, Enteropathy, Deafness, Neuropathy, Icthyosis, Keratodermia

ZINC

- Zinc is an integral component of many metalloenzymes in the body
- It is involved in the synthesis and stabilization of proteins, DNA, and RNA and plays a structural role in ribosomes and membranes
- Zinc is necessary for the binding **of steroid hormone receptors** and several other transcription factors to DNA
- Zinc is absolutely required for normal spermatogenesis, fetal growth, and embryonic development.

Zn Deficiency

- Mild chronic zinc deficiency can cause stunted growth in children, decreased taste sensation (hypogeusia), impaired immune function
- Severe chronic zinc deficiency can cause hypogonadism, dwarfism, hypopigmented hair.

Acrodermatitis enteropathica

- Rare autosomal recessive disorder characterized by abnormalities in zinc absorption
- Clinical manifestations include diarrhea, alopecia, muscle wasting, depression, irritability, and a rash involving the extremities, face, and perineum
- The rash is characterized by vesicular and pustular crusting with scaling and erythema
- The diagnosis of zinc deficiency is usually made by a serum zinc level < 12 mol/L (< 70 g/dL).

Zn Toxicity

- Acute zinc toxicity after oral ingestion causes nausea, vomiting, and fever
- Zinc fumes from welding may also be toxic and cause fever, respiratory distress, excessive salivation, sweating, and headache.

SELENIUM

- Selenium, in the form of selenocysteine, is a component of the all the enzymes that contain Selenocysteine
- Selenium is being actively studied as a chemo-preventive agent against certain cancers, such as prostate cancer.

Keshan disease

An endemic cardiomyopathy found in children and young women residing in regions of China where dietary intake of selenium is low (< 20 g/d).

Selenium toxicity (Kashinbeck Disease)

Chronic ingestion of high amounts of selenium leads to selenosis **(Kashinbeck Disease)**

It is characterized by hair and nail brittleness and loss, **garlic breath odor** (Due to Dimethyl selenide), skin rash, myopathy, irritability, and other abnormalities of the nervous system.

CHROMIUM

- Chromium potentiates the action of insulin in patients with impaired glucose tolerance, by increasing insulin receptor–mediated signalling.

Chromium -6

- Chromium in the trivalent state is found in supplements and is largely nontoxic
- Chromium-6 is a product of stainless steel welding and is a known pulmonary carcinogen as well as a cause of liver, kidney, and CNS damage.

FLUORIDE

- An essential function for fluoride in humans has not been described, although it is useful for the maintenance of structure in teeth and bone
- Adult fluorosis results in mottled and pitted defects in tooth enamel as well as brittle bone (skeletal fluorosis).

Minerals at a Glance

- Zinc containing protein present in the Saliva: Gusten[Q]
- Mineral stabilize hormone insulin[Q]**:** Zinc

- Mineral that potentiates action of Insulin: Chromium[Q]
- Mineral deficiency that leads to impaired Glucose tolerance: Chromium[Q]
- Highest concentration of Zn seen in Hippocampus and Prostatic Secretion
- The mineral deficiency leads to impaired Spermatogenesis: Zinc
- Garlicky odor in breath is seen in: Selenosis (Due to Dimethyl selenide)
- Selenium toxicity lead to Kaschinbeck Disease
- Low Selenium level leads to Keshan disease (Endemic Cardiomyopathy)
- Calcium dependent Cysteine Protease are called Calpain
- Calpain associated with Type II Diabetes Mellitus: Calpain 10
- Normal Blood Calcium level-9: 11 mg/dl
- Total Calcium level in the body[Q] is 1.5 kg.

Recommended Daily Allowances (RDA) of important Minerals

Mineral	RDA
Calcium[Q]	Adult-0.5g Children-1g Pregnancy and Lactation-1.5g
Iron[Q]	Males-15–20 mg Females-20–25 mg Pregnancy-40–50 mg
Iodine[Q]	150–200 µg **200–250 µg**
Phosphorus	500 mg
Magnesium	400 mg
Mangenese	5–6 mg
Sodium	5–10 g
Potassium	3–4 g
Copper	1.5–3 mg
Zinc[Q]	8–10 mg
Selenium[Q]	50–200 µg

Contd...

Other important Minerals: Functions and Deficiency manifestation

Mineral	Function	Deficiency
Cobalt	Constituent of Vitamin B12	Macrocytic Anemia
Chromium	**Potentiate the action of Insulin**	**Impaired Glucose Tolerance**
Fluoride	Constituent of Bone and teeth	Dental caries
Iodine	Thyroid Hormone Synthesis	Thyroid enlargement, ↓T4, cretinism
Molybdenum	Cofactor for Xanthine oxidase and Sulfite oxidase, Aldehyde oxidase	Severe neurologic abnormalities, Xanthinuria
Selenium	Cofactor for Glutathione Peroxidase Deiodinase, Thioredoxin Reductase Antioxidant along with Vitamin E	**Keshan's Disease (Cardiomyopathy),** heart failure, striated muscle degeneration
Zinc	Cofactor for **Carbonic Anhydrase Carboxy Peptidase Lactate Dehydrogenase Alcohol Dehydrogenase Alkaline Phosphatase**	Growth retardation, ↓**taste** and smell, alopecia, dermatitis, diarrhea, **immune dysfunction**, failure to thrive, **gonadal atrophy,** congenital malformation Impaired wound healing
Mangenese	Cofactor for Arginase, Carboxylase, **Kinase**, Enolase, Glucosyl Transferase, PhosphoGlucoMutase Required for RNA Polymerase	Impaired growth and skeletal development, reproduction, lipid and carbohydrate metabolism; upper body rash

REVIEW QUESTIONS

Fat Soluble Vitamins

1. In the crystalline lens, level of tocopherol and Ascorbate is maintained by: (AIIMS May 2014)

a. Glutathione
b. Glyoprotein
c. Fatty Acid
d. Glucose

Ans. a. Glutathione *(Ref: Harper 30/e p546)*

2. All are true about vitamin D metabolism, except: (AIIMS Nov 2011)

a. 1-alpha hydroxylation occurs in kidney
b. 25-alpha hydroxylation occurs in Liver
c. In absence of sun light, the daily requirement is 400–600 IU per day
d. Williams syndrome is associated with mental retardation, precocious puberty and obesity

Ans. d. Williams Syndrome is associated with mental retardation, precocious puberty and obesity

3. Vitamin K is required for: **(AIIMS Nov 2007)**

a. Hydroxylation
b. Chelation
c. Transamination
d. Carboxylation

Ans. d. Carboxylation *(Ref: Harper 30/e p554)*

4. Vitamin A intoxication cause injury to:

a. Lysosomes **(AIIMS Nov 2006)**
b. Mitochondria
c. Endoplasmic reticulum
d. Microtubules

Ans. a. Lysosomes

5. Active form of Vitamin D is: **(AIIMS Nov 2006)**

a. Cholecalciferol
b. 24,25(OH)2vit-D
c. 1,25(OH)2vit-D
d. 25-OH vit-D

Ans. c. 1, 25 (OH)2Vit D

This is otherwise called calcitriol

6. Which of these has antioxidant properties?

a. Tocopherol **(PGI Nov 2012)**
b. Reduced Glutathione
c. Citrulline
d. Lycopene

Ans. a, b, d.

7. Vitamin K is involved in the post-translational modification of: **(AIIMS Nov 08, May 01, AIPGME 2011)**

a. Glutamate
b. Aspartate
c. Lysine
d. Proline

Ans. a. Glutamate *(Ref: Harper 30/e p554)*

8. Which Vitamin is required for carboxylation of clotting factors?

a. Vitamin A
b. Vitamin D
c. Vitamin E
d. Vitamin K

Ans. d. Vitamin K *(Ref: Harper 30/e p554)*

Vitamin K is required for the post-translational carboxylation of glutamic acid (Gamma Carboxylation), which is necessary for calcium binding to γ carboxylated proteins.

9. All the following have antioxidant action except: **(Kerala 2011)**

a. Vitamin A
b. Vitamin E
c. Selenium
d. Vitamin D

Ans. d. Vitamin D

- Adequate Selenium intake maximize the antioxidant action of Glutathione Peroxidase
- So, Selenium is also considered as a compound having antioxidant action
- Nowadays Vitamin D is also considered as powerful natural membrane antioxidant
- But as it is an old question, Vitamin D is the best answer.

10. Which of the following is true about vitamin K? **(NBE pattern Q)**

a. Vit K dependent factors undergo post-transcriptional modification
b. Prothrombin is a vitamin K dependent factor
c. Stuart-Prower factor is not vitamin K dependent
d. Menadione is a natural water insoluble vitamin K used in clinical practice

Ans. b. Prothrombin is a vitamin K dependent factor

- Vitamin K helps Post-translational modification
- Stuart prower factor is Factor X, which is not Vitamin K dependent
- Prothrombin is factor II, so it is Vitamin K dependent
- Menadione is Synthetic Vitamin K.

11. Vitamin E deficiency causes all except:

a. Ataxia **(NBE pattern Q)**
b. Areflexia
c. Ophthalmoplegia
d. Neuropathy

Ans. c. Ophthalmoplegia

Principal Clinical Findings of Vitamin Malnutrition

Nutrient	Clinical finding
Thiamin	**Peripheral nerve damage** (beriberi) or central nervous system lesions (Wernicke-Korsakoff syndrome)

Contd...

Nutrient	Clinical finding
Riboflavin	Magenta tongue, angular stomatitis, cheilosis, **seborrheic dermatitis**
Niacin	Pellagra: pigmented rash of sun-exposed areas (photosensitive dermatitis), bright red tongue, diarrhea, apathy, memory loss, disorientation, depressive psychosis
Vitamin B6	Seborrhea, glossitis, convulsions, neuropathy, depression, confusion, microcytic anemia
Folate	Megaloblasticanemia, atrophic glossitis, depression, ↑homocysteine
Vitamin B12	Pernicious anemia = megaloblasticanemia with **degeneration of the spinal cord**, loss of vibratory and position sense, abnormal gait, **dementia[Q]**, impotence, loss of bladder and bowel control, **↑homocysteine, ↑methylmalonic acid**
Pantothenic Acid	Peripheral nerve damage (nutritional melalgia or 'burning foot syndrome')
Vitamin C	Scurvy: petechiae, ecchymosis, coiled hairs, inflamed and bleeding gums, joint effusion, poor wound healing, fatigue
Vitamin A	Xerophthalmia, night blindness, Bitot's spots, follicular hyperkeratosis, impaired embryonic development, immune dysfunction
Vitamin D	Rickets: skeletal deformation, rachitic rosary, bowed legs; osteomalacia
Vitamin E	Peripheral neuropathy, spinocerebellar ataxia, skeletal muscle atrophy, retinopathy
Vitamin K	Elevated prothrombin time, bleeding

12. Which coenzyme act as reducing agent in anabolic reaction? (NBE pattern Q)

a. $FADH_2$
b. $FMNH_2$
c. NADPH
d. NADH

Ans. c. NADPH

NADPH is used in reductive biosynthesis of Fatty acids, Steroids, etc. So it is used for anabolic reactions.

13. Most powerful chain breaking antioxidant: (NBE pattern Q)

a. Glutathione peroxidase
b. Alpha tocopherol
c. Superoxide dismutase
d. Vitamin C

Ans. b. Alpha Tocopherol

Antioxidants fall into two classes:

1. Preventive antioxidants, which reduce the rate of chain initiation. They are Glutathione Peroxidase, Catalase
2. Chain-breaking antioxidants, which interfere with chain propagation. They are Superoxide Dismutase, Uric Acid, Vitamin E (Most powerful).

Water Soluble Vitamins

14. Biotin act as a coenzyme for all except: (AIIMS Nov 2015)

a. Pyruvate to Oxaloacetate
b. Acetyl CoA to Malonyl CoA
c. Propionyl CoA to Methyl Malonyl CoA
d. Glutamate to Gamma Carboxy Glutamate

Ans. d. Glutamate to Gamma Carboxy Glutamate *(Ref: Harper 30/e p561)*

Biotin transfer CO_2 in

- Acetyl CoA Carboxylase
- Pyruvate Carboxylase
- Propionyl CoA carboxylase
- Methyl Crotonyl CoA carboxylase.

15. Vitamin B12 is not required for: (AIIMS Nov 2015)

a. Glycogen Phosphorylase
b. Methionine Synthase
c. Methyl malonyl CoA Mutase
d. Leucine Amino Mutase

Ans. a. Glycogen Phosphorylase *(Ref: Harper 30/e p558)*

Coenzyme Role of Cobalamin

- L Methyl Malonyl CoA $\xrightarrow{\text{Methyl Malonyl CoA Mutase}}$ Succinyl CoA
- Homocysteine $\xrightarrow{\text{Methionine Synthase or Homocysteine Methyl Transferase}}$ Methionine
- Leucine Amino Mutase.

16. A vitamin derived from amino acid is: (JIPMER Nov 2015)

a. Biotin
b. Pantothenic acid
c. Niacin
d. Folic acid

Ans. c. Niacin *(Ref: Harper 30/e p556)*

Niacin is strictly not a vitamin as it can be synthesized from Tryptophan.

17. Vitamin for which RDA is based on protein intake is: (JIPMER Nov 2015)

a. Niacin
b. Riboflavin

c. Pyridoxine
d. Thiamine

Ans. c. Pyridoxine

- The RDA of Pyridoxine is dependent on Protein intake
- The RDA of Thiamine is dependent of Carbohydrate intake.

18. Megaloblastic anemia seen in: **(JIPMER Nov 2015)**

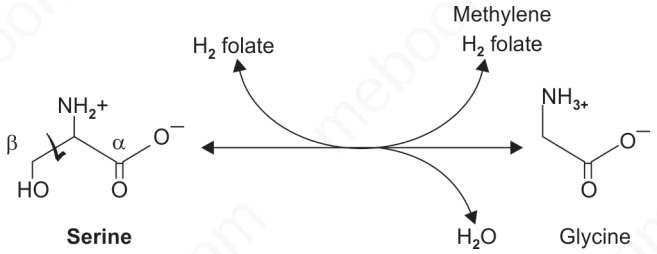
a. Ornithine Transcarbamoylase defect
b. MSUD
c. Citrullinemia
d. Orotic aciduria

Ans. d. Orotic aciduria

19. In one carbon metabolism when Serine converted to Glycine, which carbon atom is added to THFA: **(NBE pattern Q)**

a. Alpha Carbon
b. Beta Carbon
c. Delta Carbon
d. Gamma Carbon

Ans. b. Beta Carbon



20. Vitamin deficiency that cause oro oculogenital syndrome: **(APPG 2012)**

a. Vitamin B2
b. Vitamin B12
c. Zinc
d. Vitamin B3

Ans. a. Vitamin B2

21. NADPH is produced by: **(PGI May 2014)**

a. Pyruvate Dehydrogenase
b. Isocitrate Dehydrogenase
c. Succinate Dehydrogenase
d. Malate Dehydrogenase
e. αKetoGlutarate Dehydrogenase

Ans. b. Isocitrate Dehydrogenase,
d. Malate Dehydrogenase

Sources of NADPH are:

- HMP Shunt Pathway (Major source)
- Cytoplasmic Isocitrate Dehydrogenase
- Malic Enzyme (NADP Malate Dehydrogenase)

22. Vitamin deficiency causing circum corneal vascularization is: **(AIIMS 2014 May)**

a. Biotin
b. Riboflavin
c. Thiamine
d. Vitamin D

Ans. b. Riboflavin

Principal clinical findings of vitamin malnutrition

Nutrient	Clinical finding
Thiamin	Peripheral nerve damage (beriberi) or central nervous system lesions (Wernicke-Korsakoff syndrome)
Riboflavin	Magenta tongue, angular stomatitis, cheilosis, seborrheic dermatitis, circumcorneal Vascularization
Niacin	Pellagra: pigmented rash of sun-exposed areas (photosensitive dermatitis), bright red tongue, diarrhea, apathy, memory loss, disorientation, depressive psychosis
Vitamin B6	Seborrhea, glossitis, convulsions, neuropathy, depression, confusion, microcytic anemia
Folate	Megaloblasticanemia, atrophic glossitis, depression, homocysteine
Vitamin B12	Pernicious anemia = megaloblasticanemia with degeneration of the spinal cord, loss of vibratory and position sense, abnormal gait, dementia[Q], impotence, loss of bladder and bowel control, homocysteine, methylmalonic acid
Pantothenic Acid	Peripheral nerve damage (nutritional melalgia or 'burning foot syndrome')
Vitamin C	Scurvy: petechiae, ecchymosis, coiled hairs, inflamed and bleeding gums, joint effusion, poor wound healing, fatigue
Vitamin A	Xerophthalmia, night blindness, Bitot's spots, follicular hyperkeratosis, impaired embryonic development, immune dysfunction
Vitamin D	Rickets: skeletal deformation, rachitic rosary, bowed legs; osteomalacia
Vitamin E	Peripheral neuropathy, spinocerebellar ataxia, skeletal muscle atrophy, retinopathy
Vitamin K	Elevated prothrombin time, bleeding

23. False about folic acid: **(AIIMS Nov 2013)**

a. It is present in all the green leafy vegetables
b. It is proven to decrease the occurrence of neural tube defects when taken preconceptionally
c. Wheat flour in India is fortified with folate as in USA

d. Methyl folate trap is because of methionine synthase defect

Ans. c. Wheat flour in India is fortified with folate as in USA

24. Which of the vitamin deficiency lead to lactic acidosis?

a. Riboflavin
b. Thiamine
c. Niacin
d. Pantothenic acid

Ans. b. Thiamine

Thiamine deficiency affect Pyruvate Dehydrogenase, so it causes Lactic acidosis.

25. Thiamin requirement increases in excessive intake of:

a. Carbohydrate
b. Amino acid
c. Fat
d. Lecithin

Ans. a. Carbohydrate

- Thiamin requirement increases in carbohydrate intake.
- Pyridoxine (B6) requirement increases in protein intake.

26. Which of the following statement about Thiamine true? (AIIMS Nov 2008)

a. It is a coenzyme of lactate dehydrogenase
b. Its deficiency is associated with scurvy
c. Its coenzyme function is done by thiamine monophosphate
d. It is coenzyme for pyruvate dehydrogenase and a-ketoglutarate dehydrogenase

Ans. d.

It is coenzyme for pyruvate dehydrogenase and a-ketoglutarate dehydrogenase

27. Vitamin which is excreted in urine is: (AIIMS Nov 2006)

a. Vitamin A
b. Vitamin C
c. Vitamin D
d. Vitamin K

Ans. b. Vitamin C

Water soluble vitamins are excreted in urine.

28. Which of the following cannot be synthesized in the body? (PGI Nov 2012)

a. Vit K
b. Vit C
c. Thiamine
d. Riboflavin
e. Cyanocobalmin

Ans. b, c, d, e.

29. Thiamine deficiency causes decreased energy production because: (AIPGMEE 2010)

a. It is required for the process of transamination
b. It is a cofactor in oxidative reduction
c. It is a coenzyme for transketolase in pentose phosphate pathway
d. It is a coenzyme for pyruvate dehydrogenase and alpha ketoglutarate dehydrogenase

Ans. d. It is a coenzyme for pyruvate dehydrogenase and alpha ketoglutarate dehydrogenase

(Ref: Harper 30/e p555)

30. Vitamin B12 acts as coenzyme to which one of the following enzymes?

a. Isocitrate dehydrogenase
b. Homocysteine methyl transferase
c. Glycogen synthase
d. Glucose-6- Phosphate dehydrogenase

Ans. b. Homocysteine Methyl Transferase

31. Biotin is a cofactor of:

a. Carboxylase
b. Oxidase
c. Hydrolase
d. Decarboxylase

Ans. a. Carboxylase *(Ref: Harper 30/e p556)*

Coenzyme role of Biotin

Play a role in gene expression, fatty acid synthesis, gluconeogenesis and serve as a CO_2 carrier for Carboxylases enzymes and gene regulation by histone biotinylation

Coenzyme for ATP dependent Carboxylation reaction (Carbon Dioxide Fixation)

- Pyruvate Carboxylase (Pyruvate to Oxaloacetate)
- Propionyl CoA Carboxylase (Propionyl CoA to Methyl Malonyl CoA)
- Acetyl CoA Carboxylase (Acetyl CoA to Malonyl CoA)
- Methyl Crotonyl CoA Carboxylase

Biotin independent Carboxylation reaction

- Carbamoyl Phosphate Synthetase –I & II
- Addition of CO_2 to C_6 in Purine ring (AIR Carboxylase)
- Malic Enzyme (Pyruvate to Malate)

32. Post-translation modification of hydroxylysine and hydroxyproline is by: (Kerala 2009)

a. Vit C
b. Vit K
c. Vit E
d. Vit D

Ans. a. Vit C

Apart from Vitamin C, Alpha Ketoglutarate is also acting as a coenzyme in Prolyl and Lysyl Hydroxylase reaction.

33. Pantothenic acid containing coenzyme is involved in: (NBE pattern Q)

a. Decarboxylation
b. Dehydrogenation
c. Acetylation
d. Carboxylation

Ans. c. Acetylation *(Ref: Harper 30/e p561)*

Pantothenic acid as a part of CoA take part in:

- Fatty acid Oxidation
- Acetylation
- Citric acid cycle
- Cholesterol synthesis

As a part of ACP in fatty acid Synthesis

34. Vitamin given in pregnant women to prevent neural tube defect: (NBE pattern Q)

a. Folic acid
b. Vitamin B12
c. Vitamin C
d. Vitamin A

Ans. a. Folic Acid *(Ref: Harper 30/e p558)*

35. Not needed in TCA cycle: (NBE pattern Q)

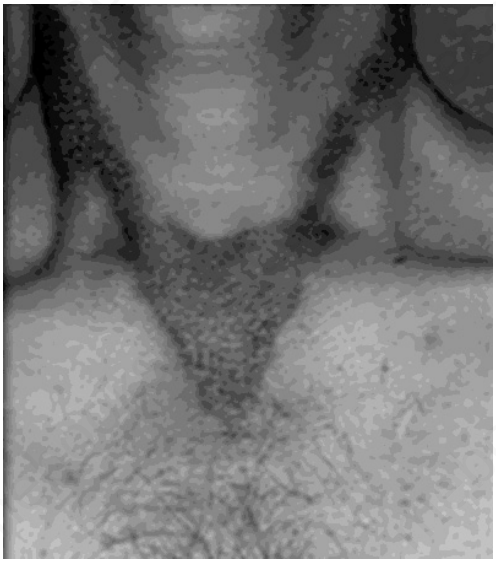
a. Pyridoxine
b. Thiamine
c. Riboflavin
d. Niacin

Ans. a. Pyridoxine

Vitamins required for TCA Cycle are Riboflavin, Niacin, Thiamin, and Pantothenic acid.

36. Identify the vitamin deficiency: (NBE pattern Q)

a. Riboflavin
b. Ascorbic Acid
c. Niacin
d. Biotin



Ans. c. Niacin

The given diagram is Casal's Necklace in Pellagra

37. Neurological worsening with anemia what is the treatment to be given: (NBE pattern Q)

a. Folic Acid alone
b. Folic acid along with Hydroxycobalamin
c. Iron
d. Pyridoxine

Ans. b.

Folic acid along with Hydroxycobalamin

38. Vitamin deficiency causing dementia: (NBE pattern Q)

a. Biotin
b. Thiamin
c. Pyridoxine
d. Vitamin B12

Ans. d. Vitamin B12

Vitamin deficiencies associated with dementia are Vitamin B1, Niacin, Vitamin B12.

39. Pantothenate Kinase associated neurodegenaration is (NBE pattern Q)

a. Wilson's Disease
b. Hallervorden- Spatz syndrome
c. McLeod Syndrome
d. LeschNyhan Syndrome

Ans. b. Hallervorden- Spatz syndrome

Pantothenate kinase associated neurodegeneration (PKAN)(formerly Hallervorden-Spatz syndrome)

- Rare autosomal recessive neurodegenerative disorder
- Chorea, dystonia, parkinsonian features, pyramidal tract features and MR
- MRI-decreased T2 signal in the globuspallidus and substantianigra, 'eye of the tiger' sign (hyperintense area within the hypointense area)
- sometimes acanthocytosis
- Neuropathologic examination indicates excessive accumulation of iron-containing pigments in the globuspallidus and substantianigra.
- Similar disorders are grouped as neurodegeneration with brain iron accumulation (NBIA)

40. The form of THFA used in treatment is: **(NBE pattern Q)**

a. N5 Formyl THFA
b. N10 Formyl THFA
c. N 5 Formimino THFA
d. N5 Methyl THFA

Ans. a. N5 Formyl THFA *(Ref: Harper 30/e p559)*

- 5 Formyl THFA is more stable than Folate, therefore used pharmaceutically
- Known as Folinic acid
- The synthetic racemic compound of Folinic acid is Leucovorin

41. Excess of avidin causes deficiency of: **(NBE pattern Q)**

a. Biotin
b. Choline
c. Vitamin B12
d. Folate

Ans. a. Biotin *(Ref: Harper 30/e p560)*

42. Thiamin act as a cofactor in: **(NBE pattern Q)**

a. Pyruvate to Oxaloacetate
b. Malonate to Oxaloacetate
c. Succinate to Fumarate
d. Pyruvate to Acetyl CoA

Ans. d. Pyruvate to Acetyl CoA *(Ref: Harper 30/e p555)*

Thiamine generally function in the decarboxylation reaction of alpha keto acids and branched chain amino acids

- **Pyruvate Dehydrogenase**Q which convert Pyruvate to Acetyl CoA.
- Alpha **KetoGlutarate Dehydrogenase**Q in Citric Acid Cycle which convert α KetoGlutarate to Succinyl CoA.
- **Branched Chain KetoacidDehydrogenase**Q which catalyses oxidative decarboxylation of Branched Chain Amino acids.
- **Trans Ketolase**Q in **Pentose Phosphate Pathway**Q. This is the biochemical basis of assay of Thiamine status of the body.

43. Sebhoreic Dermatitis is produced by deficiency of: **(NBE pattern Q)**

a. Vitamin A
b. Vitamin B1
c. Vitamin B2
d. Vitamin C

Ans. c. Vitamin B2

Riboflavin	Magenta tongue, angular stomatitis, cheilosis, **seborrheic dermatitis**

44. Severe thiamine deficiency is associated with: **(NBE pattern Q)**

a. Decreased RBC transketolase activity
b. Increased clotting time
c. Decreased RBC transaminase activity
d. Increased xanthurenic acid excretion

Ans. a. Decreased RBC Transketolase activity
Thiamin is a cofactor of Transketolase.

Minerals

45. Which of the following is wrongly matched: **(JIPMER Nov 2015)**

a. Folate-Anemia
b. Zinc-Immunodeficiency
c. Iodine –Dry Skin
d. Iron-Anemia

Ans. c. Iodine Dry skin

Mineral	Function	Deficiency
Cobalt	Constituent of Vitamin B12	Macrocytic Anemia
Chromium	**Potentiate the action of Insulin**	**Impaired Glucose Tolerance**
Fluoride	Constituent of Bone and teeth	Dental caries
Iodine	Thyroid Hormone Synthesis	Thyroid enlargement, ↓T4, cretinism
Molybdenum	Cofactor for Xanthine Oxidase and Sulfite Oxidase, Aldehyde oxidase	Severe neurologic abnormalities, Xanthinuria

Contd...

Mineral	Function	Deficiency
Selenium	Cofactor for Glutathione Peroxidase • Deiodinase, • Thioredoxin Reductase • Antioxidant along with Vitamin E	**Keshan's Disease (Cardiomyopathy),** heart failure, striated muscle degeneration
Zinc	Cofactor for • **Carbonic Anhydrase** • **Carboxy Peptidase** • **Lactate Dehydrogenase** • **Alcohol Dehydrogenase** • **Alkaline Phosphatase**	Growth retardation, ↓**taste** and smell, alopecia, dermatitis, diarrhea, **immune dysfunction**, failure to thrive, **gonadal atrophy,** congenital malformation Impaired wound healing
Mangenese	Cofactor for • Arginase, • Carboxylase, • **Kinase**, • Enolase, • Glucosyl Tranferase, • Phospho GlucoMutase Required for RNA Polymerase	Impaired growth and skeletal development, reproduction, lipid and carbohydrate metabolism; upper body rash

46. Which of the following is a non-essential metal/mineral?

a. Sodium
b. Manganese
c. Iron
d. Lead

Ans. d. Lead

Lead is a toxic mineral.

47. The 40 nm gap in between the tropocollagen molecule in collagen which serve as the site of bone formation is occupied by: (AIIMS Nov 06)

a. Carbohydrates
b. Ligand moiety
c. Calcium
d. Ferric ion

Ans. c. Calcium

48. Zinc is a cofactor for: (AIIMS Nov 2009)

a. Pyruvate Dehydrogenase
b. Pyruvate decarboxylase
c. Alphaketoglutarate Dehydrogenase
d. Alcohol Dehydrogenase

Ans. d. Alcohol Dehydrogenase

Zinc	Cofactor for • **Carbonic Anhydrase** • **Carboxy Peptidase** • **Lactate Dehydrogenase** • **Alcohol Dehydrogenase** • **Alkaline Phosphatase**

49. Cardiomyopathy is due to deficiency of (PGI Nov 2012)

a. Selenium
b. Phosphorus
c. Boron
d. Zinc
e. Iron

Ans. a. Selenium

Nutritional Causes of Cardiomyopathy are Deficiency of Thiamin, Selenium, Calcium and magnesium. Excess of Iron (Hemochromatosis)

50. Selenium deficiency causes: (PGI May 2012)

a. Dermatitis
b. Cardiomyopathy
c. Diarrhea
d. Alopecia
e. Gonadal Atrophy

Ans. b. Cardiomyopathy

51. Copper containing enzymes are: (PGI May 2012)

a. Superoxide Dismutase
b. Cytochrome Oxidase
c. Myeloperoxidase
d. Tyrosinase
e. Amino Acid Oxidase

Ans. a, b, d, e.

52. Which of the following is considered the active from of calcium:

a. Ionized Calcium
b. Albumin bound Calcium
c. Phosphate bound Calcium
d. Protein bound Calcium

Ans. a. Ionized Calcium

53. Copper involves collagen synthesis by: (Kerala 2010)

a. Lysyl oxidase
b. Lysyl hydroxylase
c. Cytochrome oxidase
d. Tyrosinase

Ans. a. Lysyl Oxidase

Cofactor role of Copper
- Amine oxidases
- Ferroxidase (ceruloplasmin) (Iron metabolism, Copper Transport)
- Cytochrome-*c* oxidase (in Complex IV of Electron Transport Chain)
- Superoxide dismutase (Free Radical Scavenging enzyme)
- Tyrosinase (Melanin Synthesis)
- Component of ferroportin (Iron Metabolism)
- Lysyl Oxidase (Cross linking in Collagen)

54. Zinc is present in: **(NBE pattern Q)**

a. Carbonic anhydrase
b. Xanthine oxidase
c. Glutathione reductase
d. Glutathione synthetase

Ans. a. Carbonic anhydrase

55. Cause of thiamine induced nerve weakness: **(NBE pattern Q)**

a. Hypocalcemia
b. Hypomagnesimia
c. Inactivation of Chloride Channel
d. Difficult to produce ACh molecules

Ans. c. Inactivation of Chloride Channel

(Ref: Harper 30/e p555)

Thiamin triphosphate has a role in nerve conduction; it phosphorylates, and so activates, a chloride channel in the nerve membrane

56. Selenium is a cofactor in the following enzyme: **(NBE pattern Q)**

a. Glutathione Peroxidase
b. Cytochrome Oxidase
c. Cytochrome Reductase
d. Xanthine Oxidase

Ans. a. Glutathione Peroxidase *(Ref: Harper 30/e p567)*

Selenium	Cofactor for
	• Glutathione Peroxidase • Deiodinase, • Thioredoxin Reductase • Antioxidant along with Vitamin E

16 Heme Metabolism and Hemoglobins

Topics Included

- Structure of Heme
- Heme Synthesis
- Porphyrias
- Heme Catabolism
- Hyperbilirubinemias
- Abnormal Hemoglobins

STRUCTURE OF HEME

Heme is a metalloporphyrin

Porphyrins

Porphyrins are cyclic compounds formed by the linkage of four pyrrole rings through methyne or methenyl bridges (= CH-)

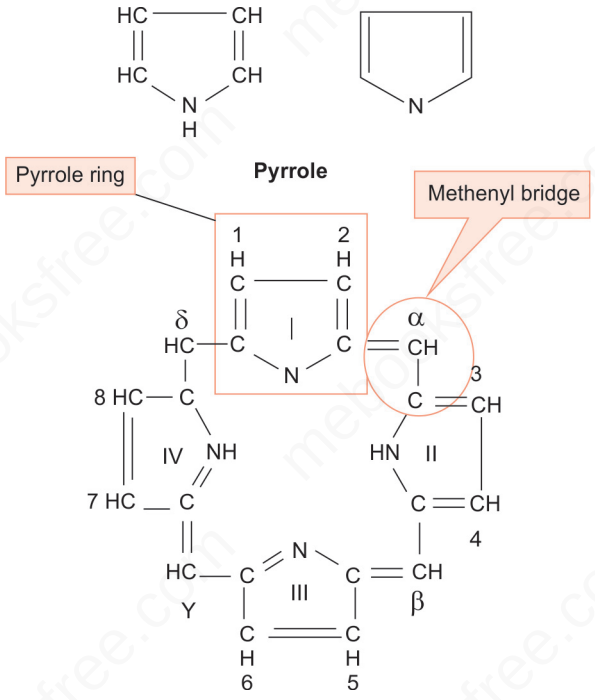


Fig. 16.1: Structure of porphyrin

Special Features of Porphyrins

- Porphyrinogens are colorless where as porphyrins are colored compounds. The conjugated double bond in the pyrrole ring and linking methenyl bridges is responsible for characteristic absorption and fluorescence spectra.
- Sharp absorption band near **400 nm called soret band** is distinguishing feature of all porphyrins after its discoverer, the French physicist Charles Soret.
- When porphyrins dissolved in strong mineral acids or in organic solvents are illuminated by ultraviolet light, they emit a strong **red fluorescence.**
- Used in cancer phototherapy because tumors often take up more porphyrins than normal tissue. The tumor is then exposed to an argon laser, which excites the porphyrins, producing cytotoxic effects. This is called Photodynamic therapy.
- Porphyrins cause photosensitivity.

Porphyrins

A characteristic property of porphyrins is the formation of complexes with metal ions bound to the nitrogen atom of the pyrrole rings.

- Iron porphyrins: Heme of hemoglobin
- Magnesium-containing porphyrin: Chlorophyll, the photosynthetic pigment of plants.

The *porphyrins* found in nature are compounds in which various *side chains* are substituted for the eight hydrogen atoms numbered in the porphyrin nucleus. The side chains are

M-Methyl A-Acetate
V-Vinyl P-Propionate

- The three important porphyrins are Uroporphyrin, Coproporphyrin and Protoporphyrin.
- The distribution of side chain in each of the above porphyrin is given in the figure (16.3) below.
- Most water soluble porphyrin is Uroporphyrin.
- Least water soluble is Protoporphyrin.
- Coproporphyrin is having intermediate water solubility.
- Heme is metal + Porphyrin.

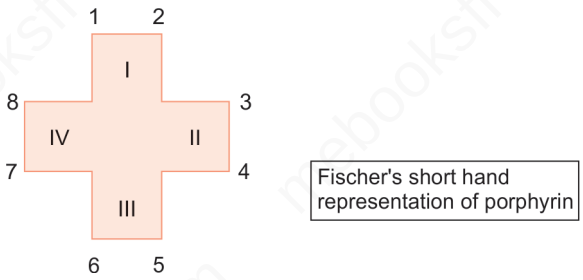


Fig. 16.2: Representation of porphyrin

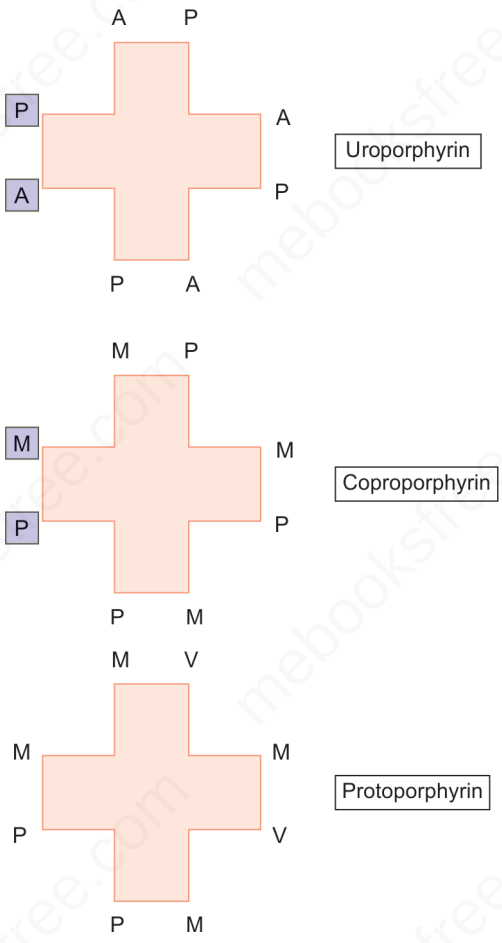


Fig. 16.3: Three types of porphyrins

- Porphyrin in heme is Protoporphyrin.
- Metal in heme is iron.
- So Heme is Ferroprotoporphyrin.
- The iron in the Heme is in Ferrous state.

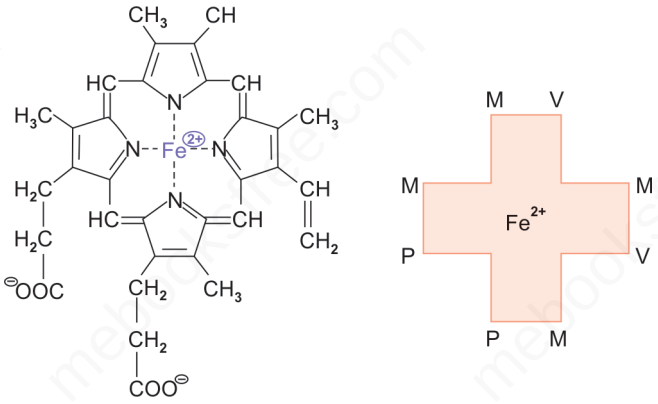


Fig. 16.4: Structure of heme

Important Heme containing Proteins	
Hemoglobin	Transport of oxygen in blood
Myoglobin	Storage of oxygen in muscle
Cytochrome *c*	Involvement in electron transport chain
Cytochrome P450	Hydroxylation of xenobiotics
Catalase	Degradation of hydrogen peroxide
Tryptophan pyrrolase	Oxidation of tryptophan

BIOSYNTHESIS OF HEME

- Site: Synthesized in almost all tissues in the body EXCEPT in mature erythrocytes.
- 85% in erythroid Precursor cells in the bone marrow and majority of remainder in hepatocyte
- Organelle: Partly cytoplasmic and partly mitochondrial
- Starting Materials: Succinyl CoA and Glycine.

Steps of Synthesis of Heme

Can be divided into

- Synthesis of Porphobilinogen (Monopyrrole)
- Synthesis of Uroporphyrinogen (Tetrapyrrole)
- Conversion of Uroporphyrinogen to Protoporphyrin
- Formation of Heme by incorporation iron.

Synthesis of Porphobilinogen (Monopyrrole)

ALA Synthase (ALAS): Catalyze condensation reaction between succinyl-CoA and glycine to form-**α amino-β-ketoadipic acid,** which is rapidly decarboxylated to form **-δ aminolevulinate (ALA)**

Synthesis of ALA occurs in **mitochondria.**

Two isoforms of ALAS

- ALAS-I is expressed throughout the body.
- ALAS -II is expressed in erythrocyte precursor cells.
- ALAS-1 is the rate limiting step of hepatic heme synthesis. Heme acts as the negative regulator of ALAS-1. ALAS-1 is induced by the drugs whose metabolism require Cytochromes.
- ALAS-2 is not feedback regulated by heme. ALAS-2 is not induced by drugs.

ALA Dehydratase

- Two molecules of ALA are condensed by the enzyme **ALA dehydratase** to form two molecules of water and one mol of **porphobilinogen** (PBG) Thus the first precursor monopyrrole is formed.
- Takes place in the **cytosol.**
- ALA dehydratase is a zinc-containing enzyme
- This enzyme is sensitive to inhibition by **lead,** as can occur in lead poisoning.

Synthesis of Uroporphyrinogen (Tetrapyrrole)

The formation of a* cyclic tetrapyrrole *—i.e. a porphyrin— occurs by condensation of four molecules of PBG.

Uroporphyrinogen–I Synthase or HMB Synthase or PBG Deaminase

- Four molecules of PBG condense in a head-to-tail manner to form a linear tetrapyrrole, **hydroxymethylbilane** (HMB).
- 4 mols of NH_3 is released. Takes place in the cytosol.
- The reaction is catalyzed by **uroporphyrinogen I synthase,** also named PBG deaminase or HMB synthase.

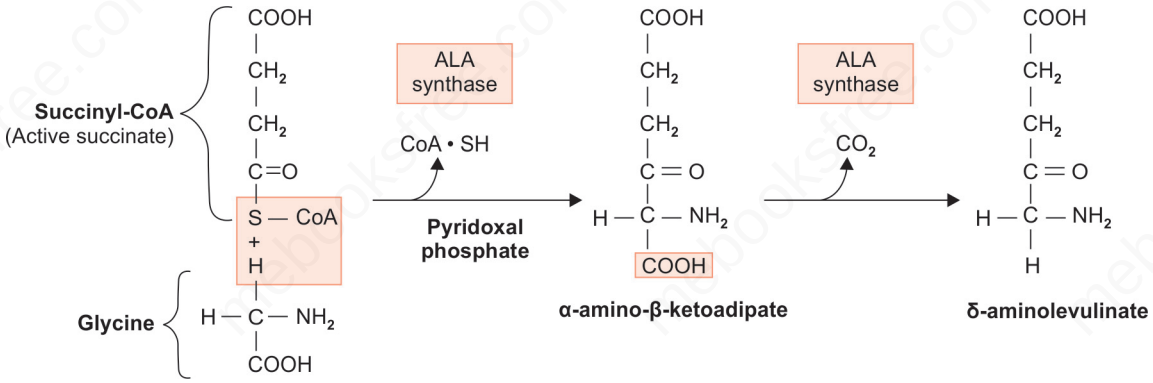


Fig. 16.5: Synthesis of ALA

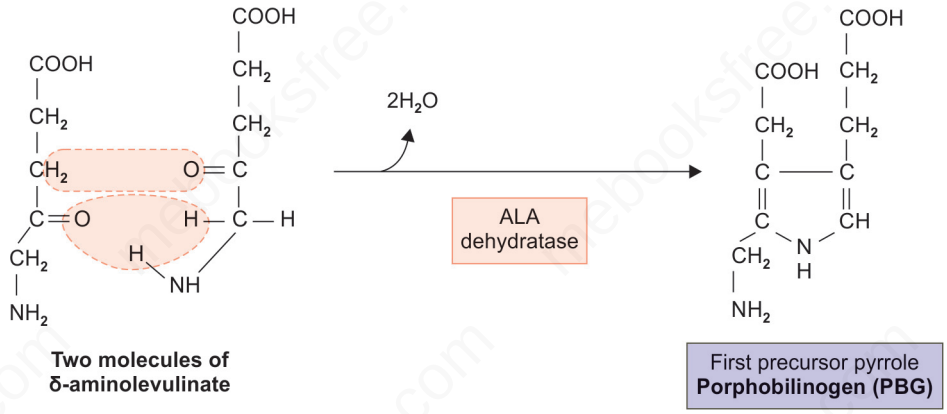


Fig. 16.6: Synthesis of PBG

Uroporphyrinogen III synthase

- HMB is converted to Uroporphyrinogen III by Uroporphyrinogen III synthase.
- Uroporphyrinogen is thus the first porphyrin precursor formed.
- Under normal conditions, the uroporphyrinogen formed is almost exclusively the III isomer.
- But in certain porphyrias, HMB cyclizes spontaneously to form **uroporphyrinogen I.**

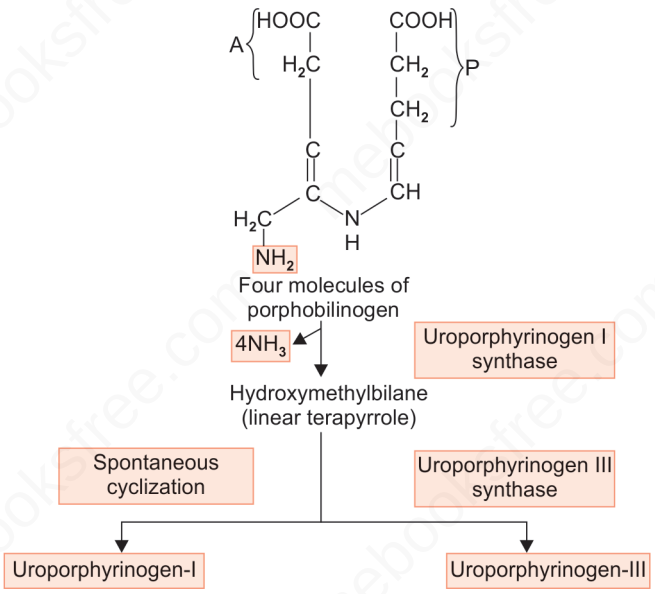


Fig. 16.7: Synthesis of Uroporphyrinogen

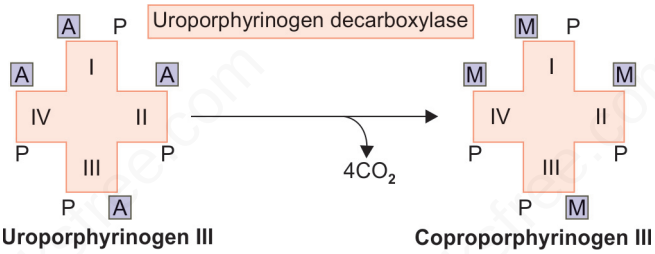


Fig. 16.8: Synthesis of Coproporphyrinogen

Conversion of Uroporphyrinogen to Protoporphyrin by Uroporphyrinogen Decarboxylase

- Uroporphyrinogen III is converted to **coproporphyrinogen III** by decarboxylation of all of the acetate (A) groups, which changes them to methyl (M) substituent.
- The reaction is catalyzed by **uroporphyrinogen decarboxylase.**
- This is also capable of converting uroporphyrinogen I to coproporphyrinogen I
- This also takes place in the cytosol
- Coproporphyrinogen III then enters the mitochondria, where it is converted to **protoporphyrinogen III**
- The **mitochondrial** enzyme **coproporphyrinogen oxidase** catalyzes the decarboxylation and oxidation of two propionic side chains to form protoporphyrinogen.
- This enzyme is able to act only on type-III coproporphyrinogen, which would explain why type I protoporphyrins do not generally occur in nature.

Protoporphyrinogen Oxidase

The oxidation of protoporphyrinogen to **protoporphyrin** is catalyzed by another **mitochondrial** enzyme, **protoporphyrinogen oxidase**.

Formation of Heme by incorporation of Iron

- This is the final step in heme synthesis
- It involves the incorporation of ferrous iron into protoporphyrin in a reaction
- This step is catalyzed by **ferrochelatase (heme synthase)**
- Takes place in the mitochondria

Regulation of Heme Synthesis

- ALA Synthase^{Q} is the key regulatory enzyme in hepatic biosynthesis of Heme
- **ALA synthase** occurs in both **hepatic** (ALAS1) and **erythroid** (ALAS2) forms.

The rate-limiting reaction in the synthesis of heme in liver is ALAS1.

It appears that **heme,** probably acting through an aporepressor molecule, acts as **a negative regulator** of the synthesis of ALAS1 by repression-derepression mechanism.

Thus, the rate of synthesis of ALAS1 increases greatly in the absence of heme and is diminished in its presence.

Factors that Affect Heme Synthesis

- *Drugs:* that induce Hepatic Cytochromes, e.g. Barbiturates^{Q}, Griseofulvin
- *Lead*^{Q}: Inhibit steps catalyzed by ALAdehydratase and ferrochelatase
- *INH:* Decrease availability of PLP.

Basis of administration of glucose to relieve acute attacks of porphyrin

High cellular concentration of glucose prevents induction of ALA Synthase.

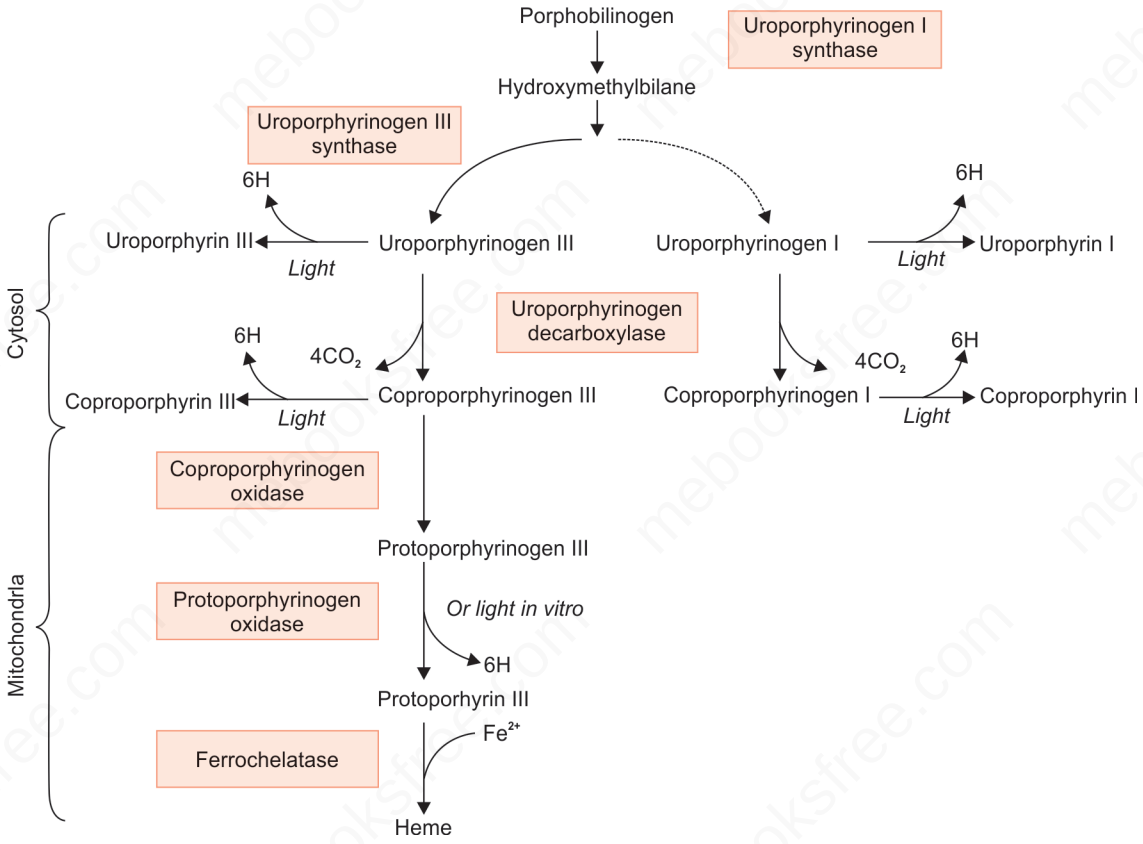


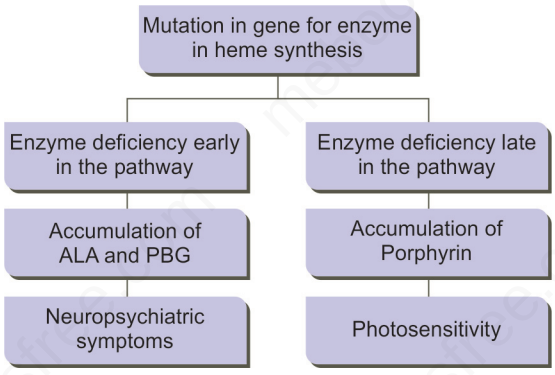
Fig. 16.9: Summary of conversion of PBG to heme

PORPHYRIAS

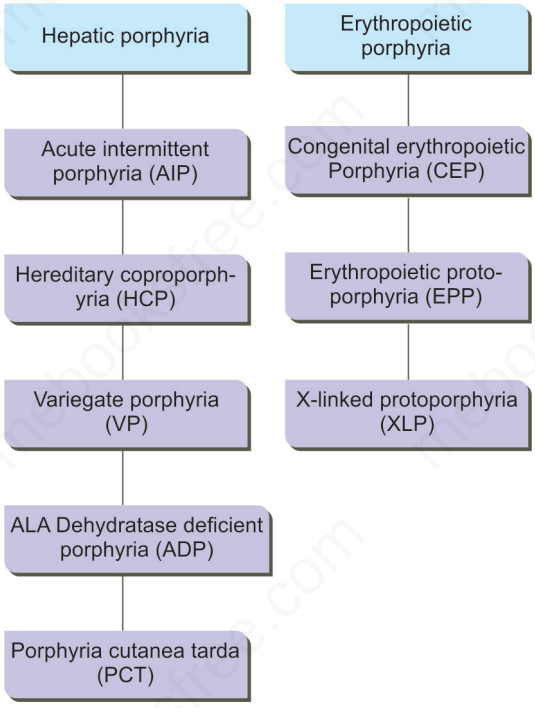
The *porphyrias* are a group of disorders due to abnormalities in the pathway of biosynthesis of heme; they can be *genetic* or *acquired*.

Most of the porphyrias are inherited autosomal dominant EXCEPT
- ALAD enzyme Deficiency [ADP]
- Congenital Erythropoeitic Porphyria [CEP]
- Erythropoetic Protoporphyria [EPP]
- X Linked Protoporphyria [XLP]

Concept of Clinical features of Porphyria



Classification of Porphyrias



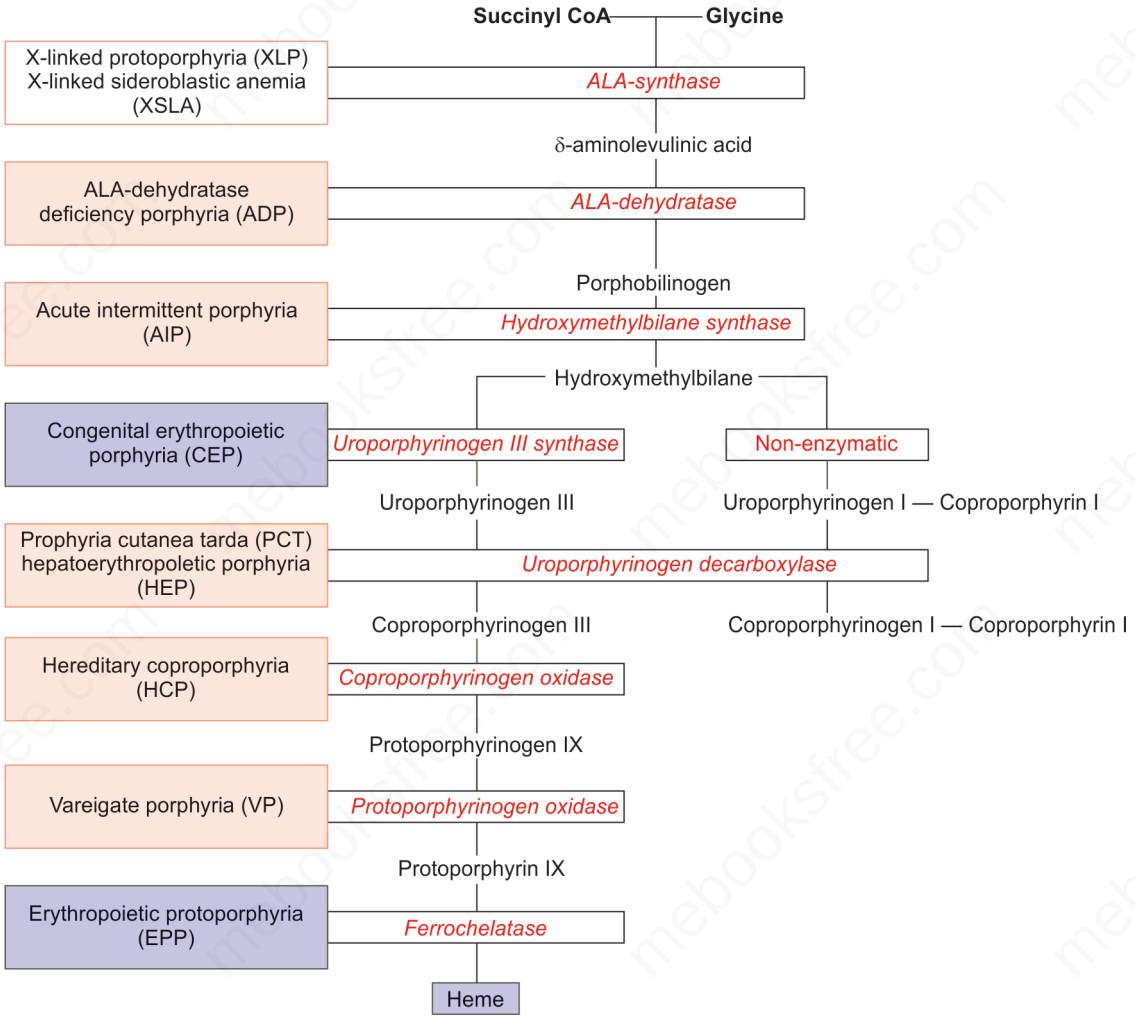


Fig. 16.10: Biochemical defects of various porphyrias

Porphyrias at a glance

- Erythropoietic porphyrias usually present with Cutaneous photosensitivity.
- Hepatic Porphyria with Cutaneous photosensitivity is Porphyria Cutanea Tarda (PCT).
- Hepatic Porphyria is symptomatic in adults.
- Erythropoietic Porphyria usually present at birth or early childhood.
- Porphyria that presents as Nonimmune Hydrops Fetalis is Congenital Erythropoietic Porphyria.
- Most common Porphyria is PCT.
- Most common acute Porphyria is Acute Intermittent Porphyria (AIP)

Contd...

Contd...

- Most common Porphyria in children is Erythropoietic Protoporphyria (EPP)
- Porphyria which can be sporadic is Porphyria Cutanea Tarda (PCT)
- Porphyria that is frequently seen in countries where Hepatitis C and HIV is prevalent is PCT
- Confirmatory diagnostic test for all Porphyrias are Enzyme analysis and Mutation analysis.
- First line investigation of Porphyrias with neurovisceral symptoms is Spot Urine ALA and PBG.
- First line investigation of Porphyrias with Photosensitivity is Plasma Porphyrins.

Major clinical features and laboratory features of Porphyrias

Porphyria	Deficient Enzyme	Inheritance	Principal Symptoms NV or CP+	Results of Laboratory Tests
5-ALA dehydratase-deficient porphyria (ADP)	ALA-dehydratase	AR	NV	Zn Protoporphyrin in erythrocytes
Acute intermittent porphyria (AIP)	HMB-synthase or Uroporphyrinogen I synthase	AD	NV	Urinary ALA and PBG increased

Contd...

Contd...

Porphyria	Deficient Enzyme	Inheritance	Principal Symptoms NV or CP+	Results of Laboratory Tests
Porphyria cutaneatarda (PCT)	Uroporphyrinogen decarboxylase	AD	CP	Urinary uroporphyrin I increased
Hereditary coproporphyria (HCP)	Coproporphyrinogen oxidase	AD	NV & CP	Urinary ALA, PBG, and coproporphyrin III and fecalcoproporphyrin III increased
Variegate porphyria (VP)	Protoporphyrinogen oxidase	AD	NV & CP	Urinary ALA, PBG, and coproporphyrin III and fecal protoporphyrin IX increased
Congenital erythropoietic porphyria (CEP)	Uroporphyrinogen III synthase	AR	CP	Urinary, fecal, and red cell uroporphyrin I increased
Erythropoietic Protoporphyria (EPP)	Ferrochelatase	AR	CP	Fecal and red cell protoporphyrin IX increased
X-linked protoporphyria (XLP)	ALA-synthase 2	XL	CP	Protoporphyrin in Erythrocytes and stool.

ALAD Deficient Porphyria [ADP]

- Aminolevulinic Acid Dehydratase Deficient Porphyria (ADP)
- Otherwise called Doss Porphyria
- Rare Porphyria.

Differential Diagnosis of ADP

- Lead intoxication resembles ADP as lead inhibit ALA Dehydratase and there is increased excretion of ALA.
- Hereditory Tyrosinemia Type I resembles ADP, as Succinyl Acetone resembles ALA.

Acute Intermittent Porphyria [AIP]

Defect in PBG Deaminase/HMB Synthase/ Uroporphyrinogen I Synthase

- Most common ACUTE Porphyria
- Most common symptom-Abdominal Pain
- Most common Physical Sign –Tachycardia
- Neurovisceral
- Levels of ALA and PBG increased
- Urine Red colored
- Currently liver directed gene therapy with Adeno Associated Viral vector (AAV-HMBS) has been proven to prevent drug induced AIP.
- Hepatic targeted RNA interference (RNAi) therapy directed to inhibit markedly elevated ALAS-1 mRNA, reduced the ongoing attack.

Congenital Erythropoietic Porphyria [CEP] Gunther's Disease

Uroporphyrinogen III Synthase defect

- Presents shortly after birth or in Utero as Nonimmune Hydrops
- Presents with severe Cutaneous Photosensitivity
- Porphyrins deposit in teeth and bones.
- So brownish discoloration of teeth
- Hemolysis due to erythrocyte porphyrins, hence splenomegaly
- Uroporphyrin I and Coproporphyrin I accumulate in bonemarrow, erythrocyte, plasma, urine and feces.
- Coproporphyrin I is the predominant porphyrin in feces.
- Portwine urine or Pink stain in diapers
- Erythrodontia-Reddish Flourescence of teeth when illuminated with long uv light
- Prenatal diagnosis by Porphyrin in amniotic fluid, URO Synthase enzyme activity in chorionic villus and cultured amniotic cells
- Beta Carotene Q to protect from sunlight
- Gene therapy using Human cDNA UROS retroviral vectors.

Porphyria cutanea Tarda

- Uroporphyrinogen Decarboxylase defect
- **80% is sporadic attributed to Uroporphyrinogen Decarboxylase inhibitors.**
- **The aggravating factors of PCT are**
  - Hepatitis C, HIV
  - Excess Alcohol
  - Elevated Iron
  - Estrogen
    - Most common Porphyria
    - Most readily treated Porphyria
    - Associated with Hemochromatosis
    - Blistering Skin Lesions mostly in the back of hands.
    - They are susceptible to develop chronic liver disease and are at risk for Hepatocellular Carcinoma.

Treatment

- Repeated Phlebotomy to reduce hepatic iron.
- Low dose regimen of Chloroquine or Hydroxy chloroquine.
- In patients with End stage renal Disease, administer Erythropoietin.

Erythropoietic Protoporphyria

Due to defect in Ferochelatase (FECH Mutation)

- Most common porphyria in children and second most common in adults.
- **NON BLISTERING** Photosensitivity
- Characterized by pain, swelling redness within minutes of sunlight exposure, resembling angioedema.
- Vesicular lesions are uncommon.

Diagnosis

- A substantial increase in erythrocyte protoporphyrin, which is predominantly free and not complexed with Zn is the hallmark of EPP.
- Erythrocytes exhibits red fluorescence under fluorescence microscopy at 620 nm.
- FECH mutation analysis.

Treatment

- Oral Beta Carotene may improve tolerance to sunlight.
- Afamelanotide, an alfa Melanocyte–stimulating hormone has completed phase III trials for patients with EPP and XLP.

X-Linked Protoporphyria

- *Due to increased* activity of ALA Synthase-2 due to gain of function mutation.

X-Linked Sideroblastic Anemia

- Not a Porphyria
- Due to decreased activity of ALA Synthase-2.

HEME CATABOLISM

Under normal conditions in human adults, some **200 billion erythrocytes** are destroyed per day.

A 70-kg human turns over approximately **6 g of hemoglobin** daily.

1 g of hemoglobin yields 35 mg of bilirubin[Q].

The daily bilirubin formation in human adults is approximately **250–350 mg**.

The fate of Hemoglobin

- When hemoglobin is destroyed in the body
- Globin is degraded to its constituent amino acids, which are reused.
- Iron of heme enters the iron pool, also for reuse.
- The iron-free porphyrin portion of heme is degraded, mainly in the reticuloendothelial cells of the liver, spleen, and bone marrow.

Steps of Catabolism of Heme

Site : Microsomal fraction of reticuloendothelial cells of the liver, spleen, and bone marrow.

Hemoxygenase

- Oxygen is added to the α-methyne bridge between pyrroles I and II of the porphyrin.
- Splitting of tetrapyrrole ring.
- Green pigment, Biliverdin[Q] is produced.
- **Carbon monoxide** is produced by this reaction.
- **This is the only source of endogenous CO[Q] in the body.**

Biliverdin Reductase

- Reduces the methyne bridge between pyrrole III and pyrrole IV of Biliverdin to a methylene group.
- Yellow pigment, bilirubin is produced
- This takes place in the cytosol.

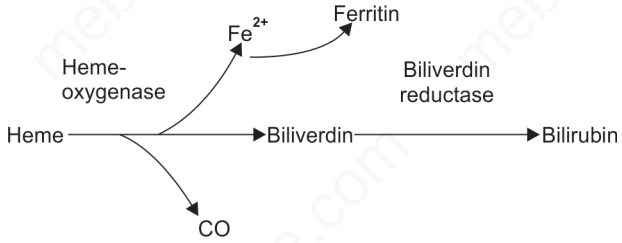


Fig. 16.11: Formation of bilirubin

Transport of Bilirubin

- Bilirubin formed in peripheral tissues is transported to the liver by plasma albumin.
- Bilirubin is only sparingly soluble in water, but its solubility in plasma is increased by noncovalent binding to albumin.
- Each molecule of albumin appears to have one high-affinity site and one low-affinity site for bilirubin.
- In 100 ml of plasma, approximately 25 mg of bilirubin can be tightly bound to albumin at its high-affinity site.
- Bilirubin in excess of this quantity can be bound only loosely and thus can easily be detached and diffuse into tissues.

Metabolism of Bilirubin

Occurs Primarily in the Liver

It can be divided into three processes.

- Uptake of bilirubin by liver parenchymal cells
- Conjugation of bilirubin with glucuronate in the endoplasmic reticulum
- Secretion of conjugated bilirubin into the bile.

Uptake of Bilirubin in the Liver

- In the liver, the bilirubin is removed from albumin
- Taken up at the sinusoidal surface of the hepatocytes by a carrier-mediated saturable system **(facilitated transport system)**
- This has a very large capacity, so that even under pathologic conditions the system does not appear to be rate limiting in the metabolism of bilirubin
- Once bilirubin enters the hepatocytes, it can **bind to certain cytosolic proteins. This is called intracellular binding.**

The proteins help in intracellular binding are

- **Ligandin** (a member of the family of glutathione *S*-transferases)
- Protein Y

Functions of intracellular binding

- They may also help to prevent efflux of bilirubin back into the blood stream.
- They help to keep bilirubin solubilized prior to conjugation

Conjugation of Bilirubin with Glucuronic Acid Occurs in the Liver

- Hepatocytes convert bilirubin to a **polar** form, which is readily excreted in the bile, by adding glucuronic acid molecules to it.
- This process is called **conjugation** and can employ polar molecules other than glucuronic acid.
- The conjugation of bilirubin is catalyzed by a specific **glucuronyltransferase.**

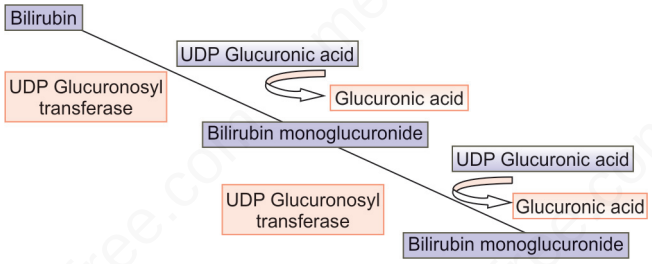


Fig. 16.12: Conjugation of bilirubin

- The enzyme is mainly located in the **endoplasmic reticulum**Q, uses UDP-glucuronic acid as the glucuronyl donor, and is referred to as bilirubin-UGT.
- Bilirubin monoglucuronide is an intermediate and is subsequently converted to the **diglucuronide.**
- Most of the bilirubin excreted in the bile of mammals is in the form of bilirubin diglucuronide.
- However, when bilirubin conjugates exist abnormally in **human plasma** (e.g. in obstructive jaundice), they are predominantly **monoglucuronides.**
- Bilirubin-UGT activity can be **induced** by a number of clinically useful drugs, including phenobarbital.

Secretion of conjugated bilirubin into the bile

- This occurs by an **active transport** mechanism
- This is the rate-limiting for the entire process of hepatic bilirubin metabolism.
- The protein involved is **MRP-2** (multidrug-resistance-like protein 2), also called multispecific organic anion transporter (MOAT).
- It is located in the **plasma membrane** of the biliary canalicular membrane.
- *Recently it is found that apart from secretion into biliary canaliculi, a portion of bilirubin diglucuronide is transported into portal circulation by MRP-3 (Multi drug Resistance associated protein-3).*
- *They are subjected to reuptake into hepatocyte by transporters, Organic anion Transporter 1B1 (OATP1B1), and 1B3 (OATP1B3).*
- It is a member of the family of ATP-binding cassette transporters.
- The hepatic transport of conjugated bilirubin into the bile is **inducible** by those same drugs that are capable of inducing the conjugation of bilirubin.

Conjugated bilirubin is reduced to urobilinogen by intestinal bacteria

- The conjugated bilirubin reaches the terminal ileum and the large intestine
- The glucuronides are removed by specific bacterial enzymes (β-glucuronidases)
- This is subsequently reduced by the fecal flora to a group of colorless tetrapyrrolic compounds called urobilinogens.

Fates of Urobilinogen

- 80–90% of the urobilinogen is converted to stercobilinogen and stercobilin by intestinal flora and excreted through feces.

- 10–20% enterohepatic circulation reaches the liver. This is called **enterohepatic urobilinogen cycle.**
- A small fraction < 3 mg/dL escape hepatic uptake, filters across renal glomerulus and is excreted through urine.

HYPERBILIRUBINEMIAS

Jaundice

- Scleral icterus indicate serum bilirubin > 3 mg/dL
- Carotenoderma can be distinguished from Icterus by sparing of sclera.

Congenital Hyperbilirubinemias

Uncojugated hyperbilirubinemias

- Gilbert's disease
- Crigler-Najjar syndrome.

Principal Differential characteristics of Crigler-Najjar and Gilbert syndrome

Features	Crigler-Najjar Syndrome		Gilbert Syndrome
	Type-I	Type-II	
Total serum bilirubin, mol/L (mg/dL)	310–755 [18–45 (usually >20)]	100–430 (usually 345) [6–25 (usually 20)]	Typically 70 mol/L (4 mg/dL)
Routine liver tests	Normal	Normal	Normal
Response to Phenobarbital	None	Decreases bilirubin by >25%	**Decreases bilirubin to normal**
Kernicterus	Usual	Rare	No
Hepatic histology	Normal	Normal	Usually normal; **increased lipofuscin** pigment
Bile characteristics			
Color	Pale or colorless	Pigmented	Normal dark color
Bilirubin fractions	>90% unconjugated	**Largest fraction (mean: 57%) monoconjugates**	Mainly diconjugates but monoconjugates increased (mean: 23%)
Bilirubin UDP-glucuronosyltransferase activity	**Typically absent**; traces in some patients	Markedly reduced: 0–10% of normal	Reduced: typically 10–33% of normal

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Features	Crigler-Najjar Syndrome		Gilbert Syndrome
	Type-I	Type-II	
Inheritance (all autosomal)	**Recessive**	**Predominantly recessive**	**7 of 8 dominant; 1 reportedly recessive**

Physiological Jaundice

- Predominantly unconjugated hyperbilirubinemia.

Causes

- Incompletely developed hepatic system
- Low UDP Glucuronyl Transferase enzyme
- Unconjugated hyperbilirubinemia that develop 2nd to 5th day of birth
- Peak level of Serum Bilirubin is 5–10 mg/dL
- Decline to normal adult concentration in 2 weeks.

Breast Milk Jaundice

- Bilirubin conjugation is inhibited by certain fatty acids that are present in the breast milk and not in serum.
- A correlation between epidermal growth factor content of breast milk and elevated bilirubin level is noted in these infants.

Lucey Driscoll Syndrome

- Transient familial neonatal hyperbilirubinemia.
- UGT1A1 inhibitor is in maternal serum, and not in breast milk unlike breast milk jaundice.

Conjugated Hyperbilirubinemias

- Dubin Johnson's syndrome
- Rotor syndrome
- Benign Recurrent intrahepatic Cholestatsis (BRIC)
- Progressive Familial intrahepatic Cholestatsis (FIC)

Dubin Johnson's Syndrome

- Autosomal recessive
- Cause–Mutation of gene encoding MRP2
- A cardinal feature is accumulation in the lysosomes of centrilobular hepatocytes of dark, coarsely granular pigment. Liver is grossly black in appearance. This pigment is thought to be an epinephrine metabolite that are not excreted properly
- Black liver jaundice
- Brom Sulphthalein test (BSP test) shows 2 Peak.

Rotor Syndrome

- Autosomal recessive
- Defective bilirubin excretion
- Recent studies indicate that deficiency of plasma transporters OATP1B1 and OATP1B3 is the cause.
- This result in reduced reuptake of conjugated bilirubin pumped into portal circulation.

Benign Recurrent Intrahepatic Cholestasis (BRIC)

- Rare disorder characterized by recurrent attacks of jaundice and pruritus.
- There are two types BRIC-1 and BRIC-2
- In BRIC-1 a gene named FIC-1 is mutated, that encodes a protein that play a role in biliary canalicular excretion of various compounds.
- In BRIC 2 mutation is in Bile salt Excretory Protein (BSEP).
- This protein is defective in Familial Intrahepatic Cholestatsis type 2 (FIC-type 2).

Progressive Familial Intrahepatic cholestasis (FIC)

FIC Type 1 (Byler's disease)

- Due to FIC-1 mutation.
- Present in early infancy as cholestasis, initially episodic.
- Unlike BRIC, Bylers disease progress to malnutrition, growth retardation and End stage Liver Disease.

FIC Type II

- Due to mutation of Bile Salt Excretory Protein (BSEP)

FIC Type III

- Due to mutation of MRP-3.

Differentiating features of important Conjugated Hyperbilirubinemia

	DJS	Rotor	PFIC1	BRIC1	PFIC2	BRIC2	PFIC3
Gene	ABCCA	SLCO1B1/SLCO1B3	ATP8B1	ATP8B1	ABCB11	ABCB11	ABCB4
Protein	MRP2	OATP1B1,OATP1B3	FIC1	FIC1	BSEP	BSEP	MDR3
Cholestasis	No	No	Yes	Episodic	Yes	Episodic	Yes
Serum γ GT	Normal	Normal	Normal	Normal	Normal	Normal	↑↑
Serum Bile acids	Normal	Normal	↑↑	↑↑ during episodes	↑↑	↑↑ during epidodes	↑↑

NB: Learn BRIC1 and PFIC1 together, then BRIC2 and PFIC2 together.

Acquired Hyperbilirubinemias

- Hemolytic Jaundice
- Hepatic Jaundice
- Obstructive Jaundice

Laboratory Tests Done to Differentiate Jaundice

Serum Bilirubin

By Van den Bergh test

- Chemical method to estimate bilirubin in serum
- Bilirubin + Ehrlich's Diazoreagent (Diazotized Sulfanilic Acid)
- Reddish purple azopigment is formed.
- They are analyzed by photometry at 540 nm

Two types of reaction in this test:

Direct and Indirect

- *Conjugated bilirubin:* Direct positive, so conjugated bilirubin is otherwise Direct bilirubin.
- *Unconjugated bilirubin:* Indirect positive, so unconjugated bilirubin is otherwise indirect bilirubin.

Tests in urine

- Urine and fecal urobilinogen-By Ehrlich's test
- **Urine Bilirubin (Bile pigment): By Modified Fouchet's test**
- **Urine Bile salt by Hay's test, Pettenkofer test.**

Liver enzyme panel

- Transaminases (AST and ALT) elevated in Hepatitis
- Alkaline Phosphatase elevated in Obstructive Jaundice
- 5′ Nucleotidase in Obstructive Jaundice.

Laboratory tests in three different types of jaundice

Condition	Serum Bilirubin	Urine Urobilinogen	Urine Bilirubin
Normal	Direct: 0.1–0.4 mg/dL	0–4 mg/24 h	Absent
	Indirect: 0.2–0.7 mg/dL		
Hemolytic-anemia	Indirect	Increased	Absent

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Condition	Serum Bilirubin	Urine Urobilinogen	Urine Bilirubin
Hepatitis	Direct and indirect	Decreased if micro-obstruction is present	Present if micro-obstruction occurs
Obstructive jaundice	Direct	Absent	Present

Delta Bilirubin or Biliprotein

- Conjugated bilirubin that is covalently linked to albumin.
- Half-life of delta bilirubin is 12–14 days [Half life of unbound Bilirubin is only 4 hrs]
- In case of conjugated hyperbilirubinemia bilirubinuria starts late.

HEMOGLOBIN

- Hemoglobin, a tetrameric protein of erythrocytes.
- Consists of a pair of α-like chains 141 amino acids long and a pair of β-like chains 146 amino acids long.
- Each globin chain enfolds a single heme moiety
- A single heme moiety, consists of a protoporphyrin IX ring complexed with a single iron atom in the ferrous state (Fe^{2+}).
- Each heme moiety can bind a single oxygen molecule.
- A molecule of hemoglobin can transport up to four oxygen molecules.

Higher Order Structure of Hemoglobin

- *Secondary structure:* Various globin chains are predominantly alpha helix.
- *Tertiary structure:* It is globular *tertiary structures* with the exterior surfaces to be rich in polar (hydrophilic) amino acids that enhance solubility, and the interior lined with nonpolar groups, forming a hydrophobic pocket into which heme is inserted
- *Quaternary structure:* It is a tetramer that contains two $\alpha\beta$ dimmers.
- The hemoglobin tetramer is highly soluble but individual globin chains are insoluble.
- Unpaired globin precipitates, forming inclusions that damage the cell.

Genetics of alpha and Beta chains

The human hemoglobins are encoded in two tightly linked gene clusters; the α-like globin genes are clustered on chromosome 16 and the β-like genes on chromosome 11.

Types of Hemoglobin

Embryonic Hemoglobins[Q]

- Hb Portland-$\zeta_2\gamma_2$
- Hb Gower I-$\zeta_2\varepsilon_2$
- Hb Gower II-$\alpha_2\varepsilon_2$

Fetal Hemoglobin[Q]

- Hb F-$\alpha 2\gamma 2$

Characteristics of Fetal Hemoglobin[Q]

- Slower Electrophoretic Mobility
- Increased resistance to Alkali Denaturation
- Decreased Interaction with 2,3 BPG.

Adult Hemoglobins

- Hb A_1-$\alpha_2\beta_2$
- Hb A_2--$\alpha_2\delta_2$

Characteristics of Hemoglobin

Hemoglobin is an allosteric Protein.

- Binding of one molecule of oxygen to one heme residue increases the affinity of binding of oxygen to other heme residue called Heme-heme interaction or Positive Cooperativity
- Hence Oxygen Dissociation Curve is Sigmoidal.

Functions of Hemoglobin

The functions of hemoglobins are to transports O_2 to the tissues and returns CO_2 and protons to the lungs.

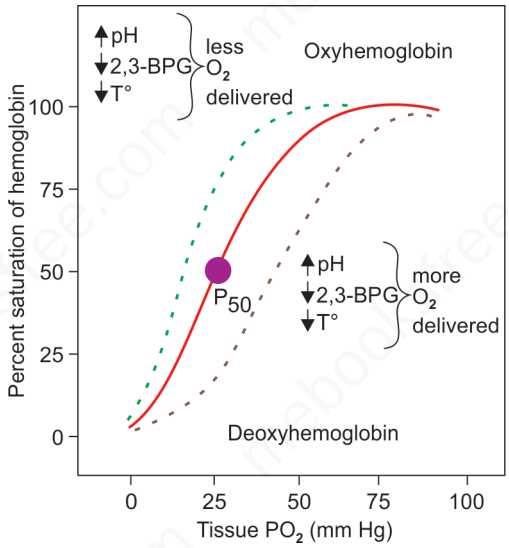
Functional Histidine Residues in Hemoglobin

- Proximal Histidine [F8] and Distal Histidine [E7] are the functional His residues in Hemoglobin.
- Fifth coordination position of iron is occupied by a nitrogen of imidazole ring of the proximal histidine, His F8.
- Distal Histidine, His E7 lies on the side of heme ring opposite, His F8.

Conformational States of Hemoglobin

- Two states are T (taut) state and R (relaxed) state
- T state is the low affinity to Oxygen state, so it is the deoxygenated state.
- T to R state is formed by breaking the salt bridges.
- R state is the high affinity to oxygen state, it is the oxygenated state.
- T state favor oxygen delivery to tissues.
- R state favor binding of Oxygen to Hemoglobin.
- Binding of first Oxygen to deoxy Hb shifts the conformation from T to R state

- Binding of 2,3 BPG stabilises the T structure of Hb, so shifts ODC to right.



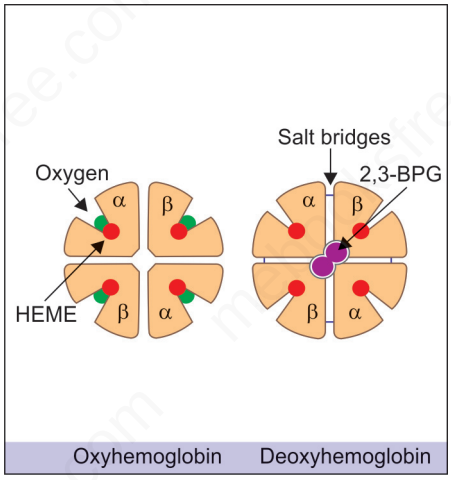


Fig. 16.13: Factors affecting the oxygen dissociation curve

Interaction of Hemoglobin with 2, 3 BPG[Q]

- 2, 3 BPG is an intermediate of Glycolysis
- Synthesized with the help of 2, 3 BPG Shunt or Rapaport Leubering Cycle.
- The Hemoglobin tetramer binds to **one mol of 2,3 BPG** in the central cavity formed **by four subunits**.
- 2,3 BPG forms salt bridges with terminal amino group of both β globin chain via, Valine, Lysine and Histidine.
- Binding of 2,3 BPG decreases the affinity of Hb to Oxygen, by stabilizing the T state.
- Shifts the ODC to right.
- 2, 3 BPG has decreased interaction with HbF.
- Because, Histidine that forms salt bridges with 2,3 BPG is not present in γ chain of HbF.
- Instead of Histidine it is Serine, so 2,3 BPG has less interaction with Fetal Hb.
- This accounts for high affinity of HbF towards Oxygen.

Glycated Hemoglobins

- These are formed **nonenzymatically** by condensation of glucose or other reducing sugars, called Glycation with alpha- and beta-chains of hemoglobin A
- Overall reaction called Maillard Reaction
- 80% glucose is added to N terminal valine of beta chain to form HbA1c
- A stands for Aldimine [Linkage between Hb and NH2 group of Valine]
- Best index of long-term control of Blood Glucose
- Since the half-life of an erythrocyte is typically 60 days, the level of glycated hemoglobin (HbA1c) reflects the mean blood glucose concentration over the preceding 6 to 8 weeks
- Normal level of HbA1c is < 5.5%
- 5.5% to 6% is good control.

HbA1c derived Average glucose value

HbA1c level	Average Whole blood glucose
5.5%	110 mg/dL
6%	126 mg/dL
7%	154 mg/dL
8%	183 mg/dL
9%	212 mg/dL
10%	240 mg/dL

Calculation of average blood glucose values from HbA1c

- Average Plasma Blood Glucose (mg/dl) = (HbA1c x 35.6) - 77.3
- Average Plasma Blood Glucose (mmol/L) = (HbA1c x 1.98) - 4.29
- Average whole blood glucose = Plasma Blood Glucose / 1.12

MYOGLOBIN

- Seen in Muscle
- Is a Monomer
- MW is 17000 KDa
- Oxygen is stored in red muscle myoglobin is released during severe exercise for use in muscle mitochondria for aerobic synthesis of ATP
- Rich in alpha helix
- Bind only one mol of Oxygen

- High affinty for Oxygen
- Exhibit no Bohr effect
- Exhibit no Cooperativity
- No interaction with 2, 3 BPG
- ODC is Hyperbolic and not sigmoidal.

HEMOGLOBINOPATHIES

Classification of Hemoglobinopathies			
	Name of Hemoglobinopathy		Altered functional or physical and chemical properties
I	**Structural hemoglobinopathies**		Amino acid sequences is altered
I A (1)	HbS, Hemoglobin Sickling	β6 Glu→Val	Abnormal Polymerization
IA (2)	HbC	β6 Glu→Lys	
I (B) Altered Oxygen affinity			
I B(1)	Hb Yakima	β99Asp→His	Increased O_2 affinity-hence polycythemia
IB(2)	Hb Kansas	β102 Asn→Lys	Low O_2 affinity—cyanosis, pseudoanemia
IC)Hemoglobin that oxidize readily			
	Hb Philly	β35Tyr→Phe	Unstable hemoglobins
	Hb Genova	β28Leu→Pro	
	Hb Koln	β98Val→Met	
	Hb M Boston		M hemoglobins Prox or Distal Histidine mutated to Tyrosine
	HbM Iwata	α87His→Tyr	
	HbM Saskatoon		
	HbM Hyde park		
	Hb M Milwaukee		
II	Thalassemias		Defective biosynthesis of globin chains
II (A)	α Thalassemias		Defective biosynthesis of α globin chains
II (B)	β Thalassemias		Defective biosynthesis of β globin chains
III	Thalassemic hemoglobin variants		Structurally abnormal Hb associated with co-inherited thalassemic phenotype
III (A)	HbE	β26 Glu→Lys	

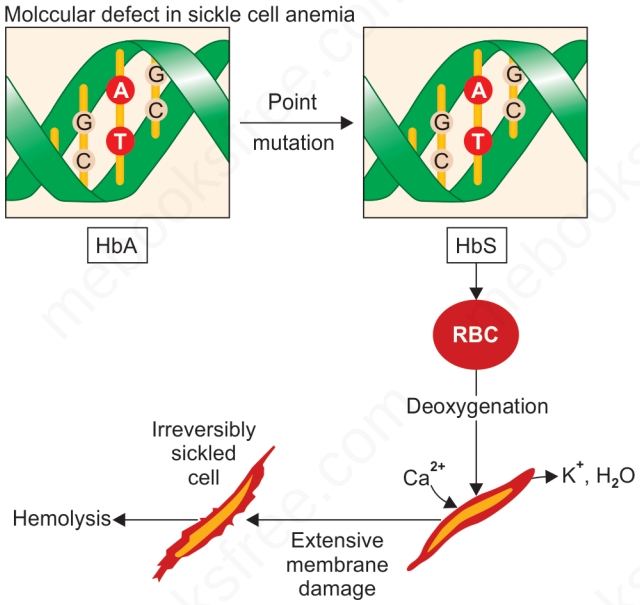
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Classification of Hemoglobinopathies			
	Name of Hemoglobinopathy		Altered functional or physical and chemical properties
III (B)	Hb Constant Spring		In the alpha chain C terminal termination codon is mutated to a coding codon hence an elongated alpha chain of 173 aminoacid is formed.
III (C)	Hb Lepore	α (δβ)2	Unequal cross over and recombination even that fuses proximal end of δ gene with distal end of β gene.
IV	Hereditary persistence of fetal hemoglobin		Persistence of high levels of HbF into adult lif

Sickle Cell Anemia

Molecular Defect in Sickle cell anemia



Mutations in Sickle cell syndromes are:

- A partially acceptable missense mutation in sequence of codon 6 of β globin chain.
- GAG to GTG, So A to T mutation hence an example of transversion.
- The sixth amino acid in β globin chain is changed from glutamic acid to valine
- A polar amino acid is replaced by a nonpolar amino acid, so an example of Nonconservative mutation.

Pathological changes due to mutation are:

- HbS **polymerizes reversibly** when deoxygenated to form a gelatinous network of fibrous polymers that stiffen the RBC membrane

- There is increased viscosity
- There is **dehydration** due to **potassium leakage and calcium influx**
- These changes also produce the sickle shape, or holly leaf shape
- Sickled cells **lose the pliability** needed to traverse small capillaries
- They possess altered '**sticky**' membranes that are abnormally adherent to the endothelium of small venules
- These abnormalities provoke unpredictable episodes of microvascular vasoocclusion and premature RBC destruction (hemolytic anemia).

Thalassemia Syndromes

Alpha Thalassemia Syndromes

As alpha chain is encoded by two tightly linked gene clusters and not by a single gene as in the case of β globin gene. Deletion in each gene loci is associated with a syndrome.

Condition		HbA1 %	HbH(β4) %
Silent Thalassemia	1 α gene loci deleted (-α/αα)	98–100	0
Thalassemia trait	2 α gene loci deleted (-α/-α)or(--/αα)	85–95	Rare blood cell inclusion
HbH disease	3α gene loci deleted (--/-α)	70–95	5–30

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Condition		HbA1 %	HbH(β4) %
Hydrops fetalis	All the four loci deleted	0	5–10

NB: 90–95% is Hb Barts (γ4)

Mutations in Alpha and β Thalassemia

α Thalassemia	Unequal crossing-over Large deletions Less commonly nonsense and frameshift mutations
β Thalassemia	Deletions Nonsense and frameshift mutations, Splice sites and promoter mutations

Remember
- Hb H is β4 tetramer
- Hb Barts is γ4 tetramer

Novel treatments for Hemoglobinopathies

For Sickle Cell Anemia:
- Direct gene correction in situ (Genomic editing) using Zn finger nucleases or CRISPR Cas 9
- De repressing HbF by interfering with Bcl 11a.

For Sickle cell and Thalassemia Syndromes
- Bone marrow transplantation to provide stem cells used in large number of β Thalassemia and a few cases of sickle cell anemia
- Gene therapy
- Re-establishing high level of HbF by stimulating proliferation of primitive Hb F producing progenitor cells (i.e. F cell progenitors) by Cytarabine, Hydroxy Urea, Pulsed or intermittent administration of Butyrates.

REVIEW QUESTIONS

Heme Synthesis and Porphyria

1. Heme biosynthesis do not occur in: **(AIIMS Nov 2015)**

a. Osteocyte
b. Liver
c. RBC
d. Erythroid cells of Bone marrow

Ans. c. RBC *Ref: Harper 30/e p325*

Site: Synthesized in almost all tissues in the body EXCEPT in mature erythrocytes.

85% in erythroid Precursor cells in the bone marrow and majority of remainder in hepatocyte

Organelle: Partly cytoplasmic and partly mitochondrial

Starting Materials: Succinyl CoA and Glycine.

2. In lead poisoning which of the following is seen in urine: **(AIIMS Nov 2015)**

a. Delta ALA
b. Uroporphyrin
c. Coproporphyrin
d. Protoporphyrin

Ans. a. Delta ALA

Lead inhibit ALA Dehydratase. So delta ALA increases, hence found in urine.

3. In HbS, Glutamic acid replaced by valine. What will be its electrophoretic mobility? **(AIIMS Nov 2015)**

a. Increased
b. Decreased

c. No change
d. Depends on level of concentration of HbS

Ans. b. Decreased

In HbS, Glutamic acid is replaced by Valine. Glutamic acid is negatively charged and Valine is neutral. Movement of Hb in electrophoresis depends on charge. More the negative charge, faster the mobility towards the anode. So HbS with less negative charge lags behind HbA1.

4. Which of the following porpyrias does not present with photosensitivity: (AIIMS may 2012)

a. Urophorphyrin decarboxylase
b. HMB Synthase
c. Protophophrinogen oxidase
d. Coproproporphyrinogen oxidase

Ans. b. HMB Synthase

- Porphyrias that present with Neurovisceral symptoms are Acute Intermittent Porphyria and ALA Dehydratase deficient Porphyria.
- Porphyrias that present with Cutaneous Photosensitivity are Congenital Erythropoietic Porphyria, Porphyria Cutanea Tarda, Erythropoietic Porphyria, X linked Protoporphyria.
- Porphyria that presents with neurovisceral Symptoms and Cutaneous Photosensitivity is Hereditary Coproporphyria, Variegate Porphyria.

5. A boy with staining of teeth and raised Coproporphyrin-I levels and increased risk of photosensitivity, the enzyme deficient is: (NBE pattern Q)

a. Uroporphyrinogen Synthase
b. Uroporphrinogen III Synthase
c. Uroporphyrinogen Decarboxylase
d. Coprophorphyrinogen Oxidase

Ans. a. Uroporphrinogen III Synthase

This is a case of congenital Erythropoietic porphyria Enzyme defect in CEP is Uroporphyrinogen III Synthase.

6. Acute Intermittent Porphyria is caused by: (NBE pattern Q)

a. ALA synthase
b. ALA dehydratase
c. Ferrochelatase
d. Uroporphyrinogen I synthase

Ans. d. Uroporphyrinogen I Synthase

7. Variegate porphyria enzyme defect is: (NBE pattern Q)

a. Protophorphyrin oxidase
b. Coproporphrinogen Oxidase
c. Uroporphyrinogen Decarboxylase
d. Uroporphyrinogen Synthase

Ans. a. Protoporphyrinogen Oxidase

8. No. of iron in transferrin: (NBE Pattern Q)

a. 1
b. 2
c. 3
d. 4

Ans. b. 2

Transferrin

- Transport form of Iron
- Has two iron binding state.

9. No. of iron in Ferritin: (NBE Pattern Q)

a. 4
b. 40
c. 400
d. 4000

Ans. d. 4000

Ferritin

- Storage Form of Iron
- Poly nuclear complex of Hydrous ferric oxide
- Carry about 4500 iron atoms
- Seen in Intestinal cells, Liver, Spleen and Bone marrow
- Sensitive index of body iron stores

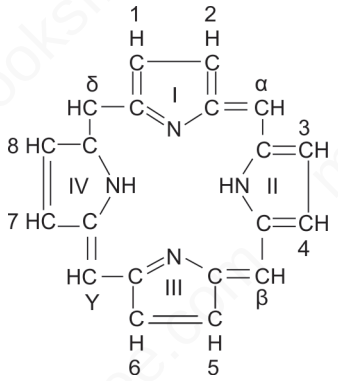
10. No of pyrrole rings in Porphyrins: (NBE Pattern Q)

a. 2
b. 3
c. 4
d. 5

Ans. c. 4

Hemoglobin

11. Identify the structure given below: (NBE pattern Q)



a. Porphyrin
b. Heme

c. Chlorophyll
d. Pyrrole

Ans. a. Porphyrin

12. 2,3 DPG binds to ..... sites in hemoglobin and causes ..... in its oxygen affinity: (AIIMS May 2014)

a. Four, increases
b. Four, decreases
c. One, increases
d. One, decreases

Ans. d. One and decreases

The Hemoglobin tetramer binds to **one mol of 2,3 BPG** in the central cavity formed **by four subunits**.

2,3 BPG forms salt bridges with terminal amino group of both β globin chain via, Valine, Lysine and Histidine Considering these two sentences, If central cavity is taken into account 2,3 BPG binds to one site.

But actually 2,3 BPG is forming salt bridges with two beta subunit, so correctly it is binding to two β sites.

So two is a better answer than four, for the number of binding site. As it is not there in the option, better answer is one.

Binding of 2,3 BPG decreases the affinity of Oxygen towards Hb

- Hb F has low affinity towards 2, 3 BPG.

Jaundice

13. A 10-year-old boy present with increased serum bilirubin, increased bilirubin in urine and no urobilinogen. Diagnosis is: (NBE pattern Q)

a. Gilbert Syndrome
b. Hemolytic jaundice
c. Viral hepatitis
d. Obstructive jaundice

Ans. d. Obstructive Jaundice

Laboratory tests in three different types of Jaundice

Condition	Serum Bilirubin	Urine Urobilinogen	Urine Bilirubin
Normal	Direct: 0.1–0.4 mg/dL	0–4 mg/24 h	Absent
	Indirect: 0.2–0.7 mg/dL		
Hemolytic anemia	Indirect	Increased	Absent
Hepatitis	Direct and indirect	Decreased if micro-obstruction is present	Present if micro-obstruction occurs
Obstructive jaundice	Direct	Absent	Present

17 TCA Cycle and Biological Oxidation

Topics Included

- TCA Cycle (Citric Acid Cycle/ Krebs Cycle)
- Shuttle Mechanisms
- Inhibitors of Electron Transport System
- High Energy Compounds
- Electron Transport Chain
- Inhibitors of Electron Transport Chain

TCA CYCLE (CITRIC ACID CYCLE/KREBS CYCLE)

Definition

- Sequence of reactions in mitochondria that oxidizes the acetyl moiety of acetyl-CoA and reduces coenzymes, that are reoxidized through the electron transport chain, linked to the formation of ATP
- The citric acid cycle is the final common pathway[Q] for the oxidation of carbohydrate, lipid, and protein
- **Site: Mitochondria.**

Overview of TCA Cycle

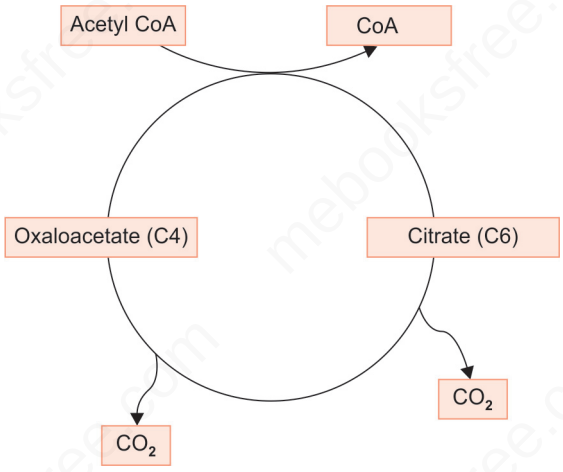


Fig. 17.1: Overview of citric acid cycle

Steps of TCA Cycle

Citrate Synthase

- Acetyl CoA + Oxaloacetate ----> Citrate
- First Tricarboxylic Acid formed is Citrate (6C)
- Irreversible Step[Q] (AIIMS June 2000)
- Citrate can cross the mitochondrial membrane and release Acetyl CoA for the synthesis of Fatty Acid by ATP Citrate Lyase.

Aconitase (Aconitate Hydratase)

- Citrate isomerized to Isocitrate
- Reversible reaction
- The reaction occurs in two steps: dehydration to cis-aconitate and rehydration to isocitrate
- Inhibited noncompetitively by Fluoroacetate[Q]
- Aconitase is a Lyase.

Isocitrate Dehydrogenase

- Isocitrate undergoes dehydrogenation catalyzed by isocitrate dehydrogenase to form, initially, oxalosuccinate
- Oxalosuccinate is decarboxylation to α –ketoglutarate (5C)
- The decarboxylation requires Mg^{2+} or Mn^{2+} ions
- First Oxidative decarboxylation
- 1 NADH is formed
- Reversible reaction.

αKetoglutarate Dehydrogenase

- αKetoGlutarate (5C) oxidised and decarboxylated to Succinyl CoA (4C)
- Second Oxidative Decarboxylatio
- 1 NADH is formed
- Physiologically Unidirectional step
- Multienzyme Complex similar to Pyruvate Dehydrogenase

5 Coenzymes of this enzyme are

1. Lipomide
2. Thiamine Pyrophosphate
3. NAD+
4. FAD
5. Coenzyme A

- Alpha KetoGlutarate Dehydrogenase noncompetitively inhibited by Arsenite[Q].

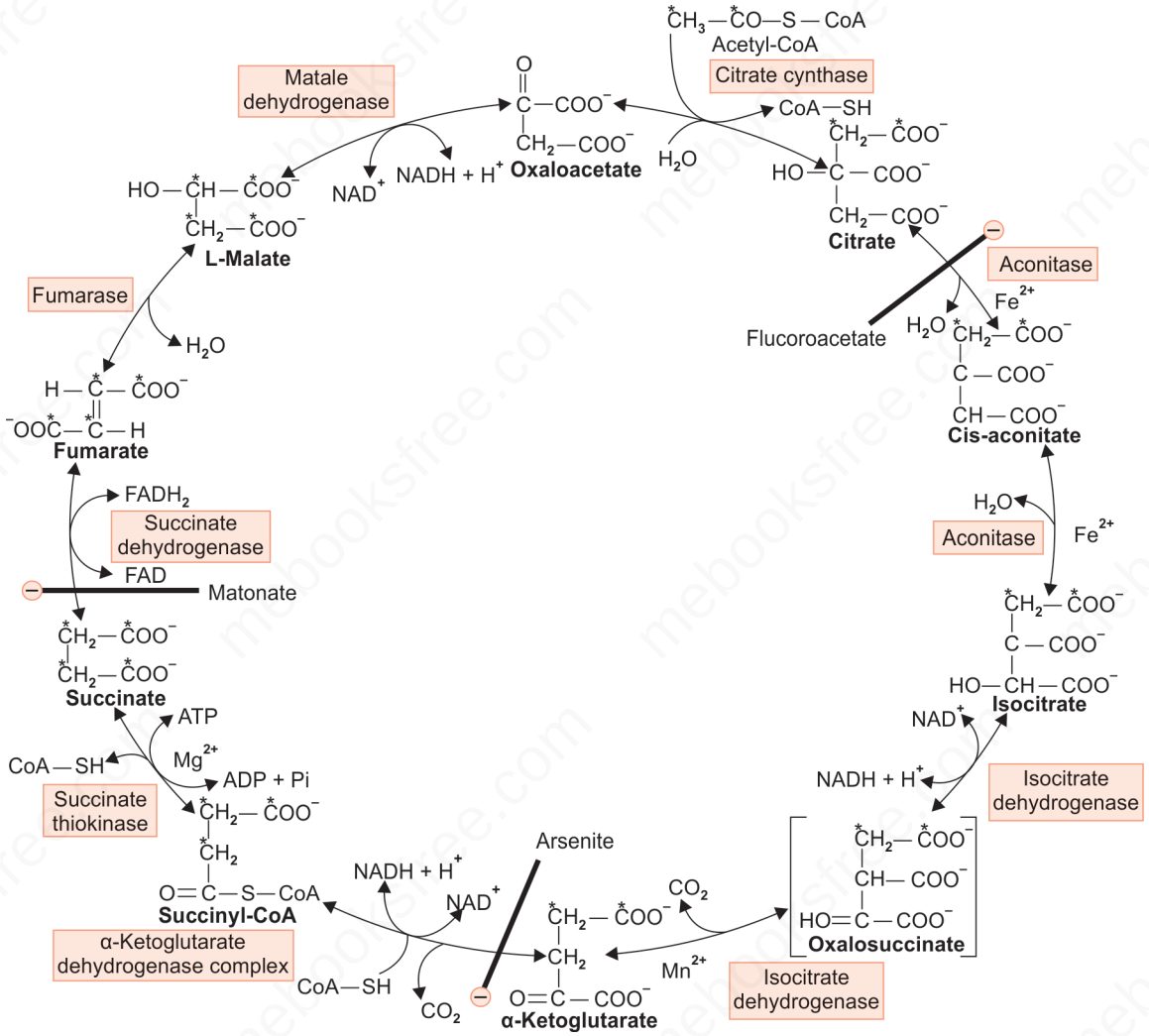


Fig. 17.2: TCA cycle

Succinate Thiokinase[Q] (Succinyl-CoA Synthetase)

- Convert Succinyl CoA to Succinate[Q]
- 1 ATP/GTP is generated
- GTP is generated in Gluconeogenic tissues like Liver and Kidney
- **Substrate Level Phosphorylation.**

Succinate Dehydrogenase

- Succinate undergo dehydrogenation reaction, forming fumarate
- The enzyme contains FAD and iron–sulfur (Fe:S) protein
- The enzyme directly reduces ubiquinone in the electron transport chain

- Only enzyme in TCA cycle attached to Inner mitochondrial Membrane
- All other enzymes are in the mitochondrial matrix
- FADH2 is formed
- Succinate Dehydrogenase is competitively inhibited by **Malonate**.Q

Fumarase (Fumaratehydratase)

- Catalyzes the addition of water across the double bond of fumarate, yielding malate
- Fumarase is a Lyase.

Malate Dehydrogenase

- Final step in TCA Cycle
- Malate is converted to Oxaloacetate
- Oxalo acetate is regenerated
- 1 NADH generated
- Oxaloacetate regenerated, hence Oxaloacetate has a catalytic role like Ornithine in Urea Cycle.

Inhibitors of TCA CycleQ

- Aconitase noncompetitively inhibited by Flouroacetate Q
- Alpha Ketoglutarate Dehydrogenase noncompetitively inhibited by ArseniteQ
- Succinate Dehydrogenase is competitively inhibited by Malonate.Q (Inhibitor of complex II of ETC).

Energetics of TCA CycleQ

Reaction	Method of ATP Production	No of ATP Generated
Isocitrate Dehydrogenase	1 NADH enter ETC	2.5 ATPs
α KetoGlutarate Dehydrogenase	1 NADH enter ETC	2.5 ATPs
Succinate Thiokinase	Substrate level Phosphorylation	1 ATP
Succinate Dehydrogenase	1 FADH2 enter ETC	1.5 ATPs
Malate Dehydrogenase	1 NADH enter ETC	2.5 ATPs
Total number of ATP per turn of TCA Cycle		10 ATPs

Three molecules of NADHQ and one of FADH2 are produced for each molecule of acetyl-CoA catabolized in one turn of the cycle.

Regulation of TCA Cycle

Regulatory steps are

- Citrate Synthase
- Isocitrate Dehydrogenase
- α Ketoglutarate Dehydrogenase
- Pyruvate dehydrogenase is also considered as the regulatory step of TCA Cycle.

Concept of regulation of TCA cycle

- High energy states inhibit TCA Cycle and vice versa
- High ATP/ADP ratio and High NADH/NAD+ ratio are inhibitors of TCA Cycle.
- High ADP and High NAD+ are activators of TCA Cycle
- Products of the pathway inhibit the regulatory enzymes

Allosteric activators and inhibitors of individual enzymes

- Long Chain Acyl CoA and ATP inhibit Citrate Synthase
- Isocitrate Dehydrogenase is inhibited by ATP and NADH
- Succinate Dehydrogenase is inhibited by Oxaloacetate
- In Muscle, the dehydrogenases of TCA Cycle are activated by Ca^{2+}, which increases during muscle contraction
- Mitochondrial Isocitrate Dehydrogenase is activated by ADP
- In a tissue such as **brain**, which is largely dependent on carbohydrate to supply acetyl-CoA, control of the citric acid cycle may occur at **pyruvate dehydrogenase**.

Remember

To answer whether a compound is an activator or inhibitor of an enzyme, think whether they are substrate or products of that enzyme/pathway

TCA Cycle is truly an Amphibolic PathwayQ

A pathway with both catabolic and anabolic role is called amphibolic pathway.

Anabolic Role of TCA Cycle

- Citrate to Fatty Acid Synthesis
- Alphaketoglutarate to GABA & Glutamate
- Succinyl CoA to Heme
- Oxaloacetate to Gluconeogenesis

Catabolic role of TCA cycle

- Acetyl CoA is completely oxidized to CO_2.

Anaplerotic Reactions of TCA CycleQ

The 6 Carbon, 5 Carbon and 4 Carbon intermediates are used for various synthetic or anabolic reactions mentioned above. So these intermediates get depleted.

To replenish these compounds filling reactions takes place. These filling up reactions are called Anaplerotic reactions.

Filling up (Anaplerotic) Reactions are at the level of Oxaloacetate (6C)

- Hydroxy Proline, Serine, Cysteine, Threonine, Glycine to Pyruvate
- Lactate to Pyruvate
- Tryptophan to Alanine to Pyruvate
- Pyruvate to Oxaloacetate by Pyruvate CarboxylaseQ, is a major filling up reaction

Contd...

Contd...

Remember

- ***Acetyl CoA is a positive Allosteric effector of Pyruvate Carboxylase***

At the level of Alpha Ketoglutarate (5C)

- Glutamine and Glutamate are the major anaplerotic substrates of Alpha Ketoglutarate

At the level of Succinyl CoA (4C)

- Valine, Isoleucine and Methionine
- Compounds that form Propionyl CoA

At the level of Fumarate (4C)

- Tyrosine and Phenyl Alanine.

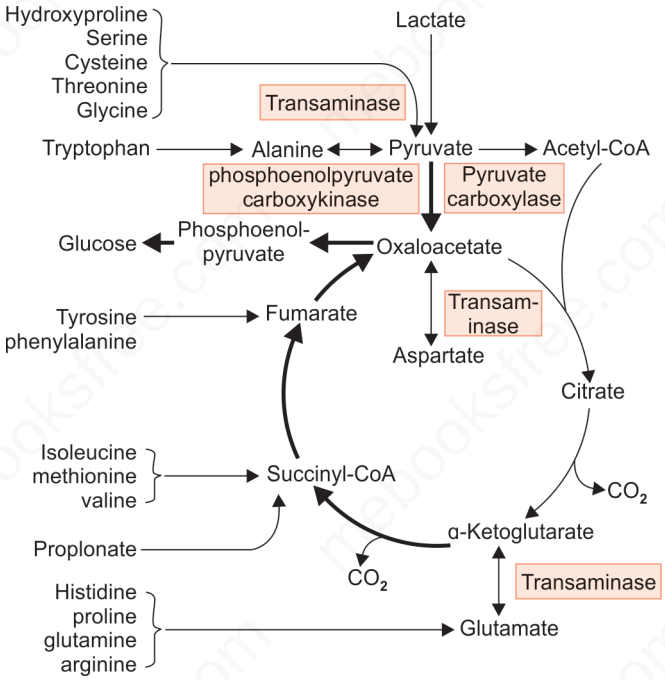


Fig. 17.3: Anaplerotic reactions of TCA Cycle

Vitamins in TCA Cycle

- Pantothenic Acid as a part of CoA
- Riboflavin as FAD
- Thiamin
- Niacin as NAD+

SHUTTLE SYSTEMS

- NADH cannot penetrate the mitochondrial membrane, but it is produced continuously in the cytosol by 3-phosphoglyceraldehyde dehydrogenase, an enzyme in the glycolysis sequence
- The transfer of reducing equivalents is carried out by using the various shuttle systems

Glycerophosphate Shuttle

This shuttle is present in some tissues (e.g., brain, white muscle), but absent in heart tissue.

The number of ATPs from 1 NADH transported to mitochondria by Glycerophosphate Shuttle

Since the mitochondrial Glycerophosphate Dehydrogenase is linked to the respiratory chain via a flavoprotein (FAD) rather than NAD, only 1.5 mol rather than 2.5 mol of ATP are formed per atom of oxygen consumed.

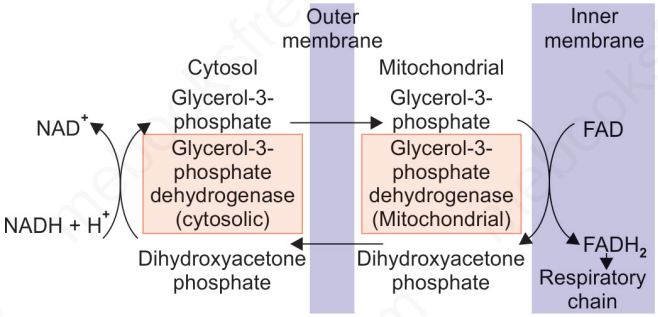


Fig. 17.4: Glycerophosphate shuttle

Malate Shuttle

Malate shuttle system is of more universal utility. Used to transport NADH from Cytosol to Mitochondria.

Reactions involved in Malate Shuttle

- NADH converted to NAD^+, Oxaloacetate to Malate
- Malate enter mitochondria via α Ketoglutarate Transporter
- Malate converted to Oxaloacetate, NADH is released
- But there is no transporter for Oxaloacetate
- Oxaloacetate react with glutamate to form aspartate and (α-ketoglutarate by Transamination)
- Aspartate and α Ketoglutarate is transported to cytosol and Oxaloacetate is reconstituted.

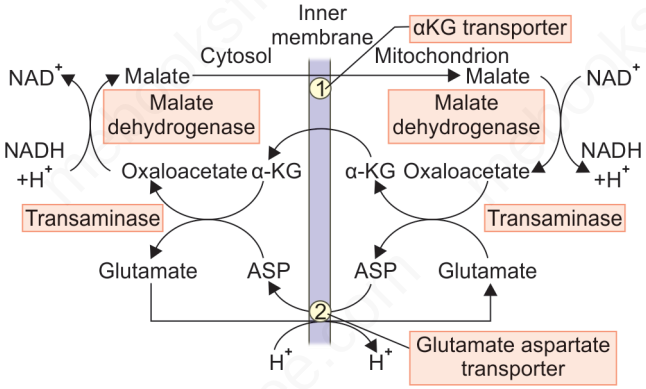


Fig. 17.5: Malate shuttle

Creatine Phosphate Shuttle

- Facilitates Transport of High-Energy Phosphate (e.g. ATP) from Mitochondria
- Sites are heart and skeletal muscle.

Reactions of Creatine Phosphate Shuttle

- ATP emerging from the adenine nucleotide transporter to intermembrane space
- CKm (An isoenzyme of **creatine kinase** (CK_m) is found in the mitochondrial intermembrane space) transfer high-energy phosphate from ATP to creatine form Creatine Phosphate
- The creatine phosphate is transported into the cytosol via protein pores in the outer mitochondrial membrane, Creatine Kinase generate extramitochondrial ATP.

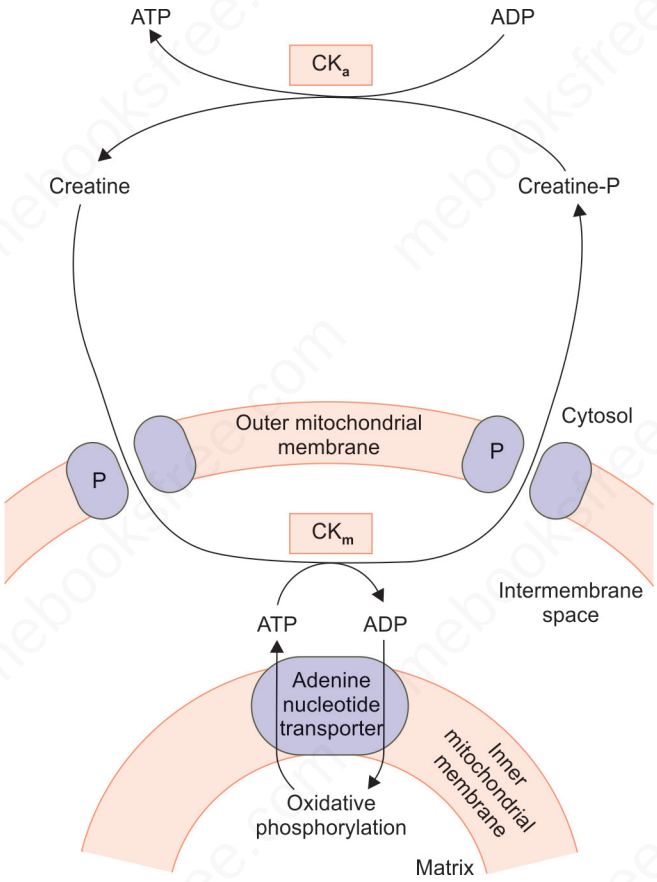


Fig. 17.6: Creatine phosphate shuttle

Redox Potential of Common Redox Couples

Electrons are transferred in the ascending order of redox couple.

Redox couple	Redox potential
H+/H2	-0.42
NAD+/NADH	-0.32
Lipoate	-0.29
Acetoacetate/β Hydroxybutyrate	-0.27
Pyruvate/Lactate	-0.19
Oxaloacetate/Malate	-0.19

Contd...

Contd...

Redox couple	Redox potential
Fumarate/Succinate	+0.03
Cyt b; Fe3+/Fe2+	+0.08
Ubiquinone(CoQ)	+0.10
Cyt c1; Fe3+/Fe2+	+0.22
Cyt a; Fe3+/Fe2+	+0.29
Oxygen/water	+0.82

Remember this table is important for national board pattern of exams. It is important to learn the order in which they are arranged, not the value of redox potential.

ELECTRON TRANSPORT CHAIN

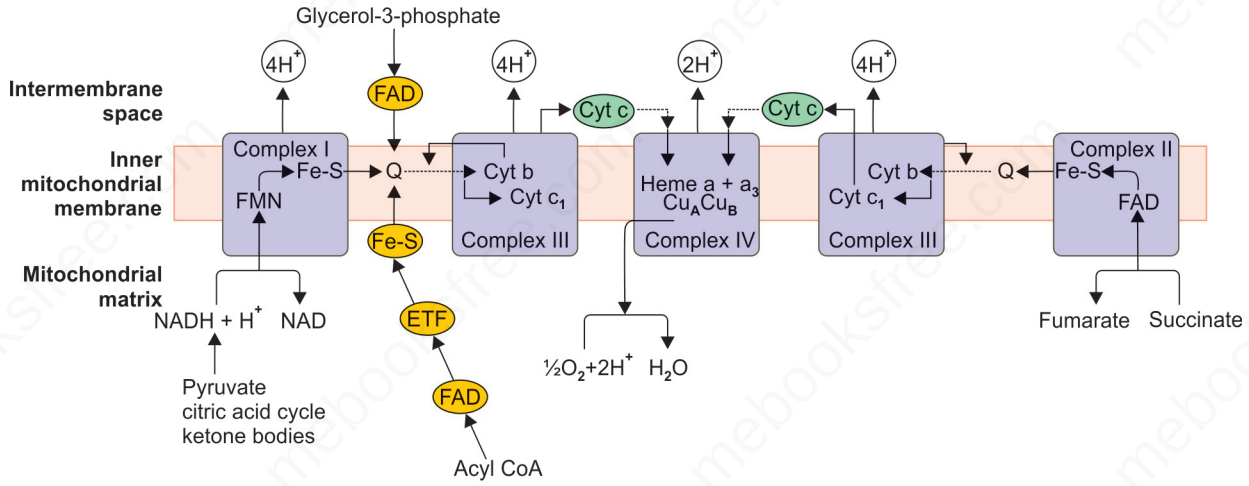


Fig. 17.7: Flow of electrons in ETC

Components of Electron Transport Chain

Site: In the Inner Mitochondrial Membrane

Components of the Electron Transport Chain *are contained in four large protein complexes*

- Complex I NADH Coenzyme Q Oxidoredutase
  - Contain FMN and Fe-S (Iron- Sulfur) Complex
  - ***Pumps 4 H+*** *to Intermembrane Space* ***(PGI Nov 09 May 10)***
- Complex II Succinate Q Reductase
  - Contain FAD and FeS Complex
  - **No H+** pumped to Intermembrane Space
- Complex III Q Cytochrome c Oxidoreductase
  - Contain Cyt b and Cyt c1
  - Contain Fe-S Complex

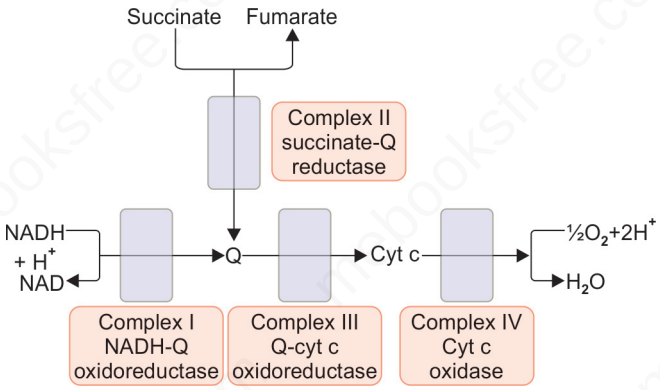


Fig. 17.8: Components of electron transport chain

Pumps ***4 H+*** *to Intermembrane Space (PGI Nov 09 May 10)*

- Complex IV Cytochrome c Oxidase
  - Contain Cyt a and a3 (now known as Heme a a3) and Copper A and Copper B centre
  - 2H+ pumped to Intermembrane Space
  - The final electron acceptor of ETC is oxygen.

Mobile Complexes in the Electron Transport Chain

Coenzyme Q

- Also called Ubiquinone
- Quinone derivative with a polyisoprenoid side chain
- Lipid solubility and small size make it a mobile electron carrier.

Cytochrome C

- Mobile electron carrier between Complex III and Complex IV
- Also play a role in programmed cell death.

OXIDATIVE PHOSPHORYLATION

The flow of electrons through the respiratory chain generates ATP by the process of oxidative phosphorylation. Oxidation coupled with Phosphorylation.

The theory behind the oxidative Phosphorylation is the Chemiosmotic theory.

The Chemiosmotic Theory

- Proposed by Peter Mitchell in 1961
- Postulates that the two processes, oxidation and Phosphorylation are coupled by a proton gradient across the inner mitochondrial membrane
- The proton motive force caused by the electrochemical potential difference (negative on the matrix side) drives the mechanism of ATP synthesis.

COMPLEX V–ATP SYNTHASE COMPLEX

- Also called as the Fifth Complex of Electron transport Chain
- The smallest molecular motor present in the human body
- Location-ATP synthase is embedded in the inner mitochondrial membrane.

Divided into two Subcomplexes

- F0 Subcomplex
- F1 Subcomplex.

F0 Subcomplex

- Hydrophobic in nature
- F0 spans the inner mitochondrial membrane
- Forms a proton channel
- Made up of a disk of 10 'C' protein subunits.

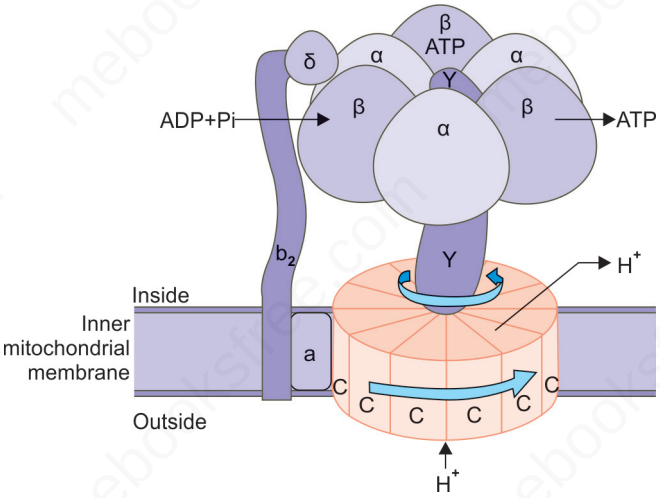


Fig. 17.9: ATP synthase

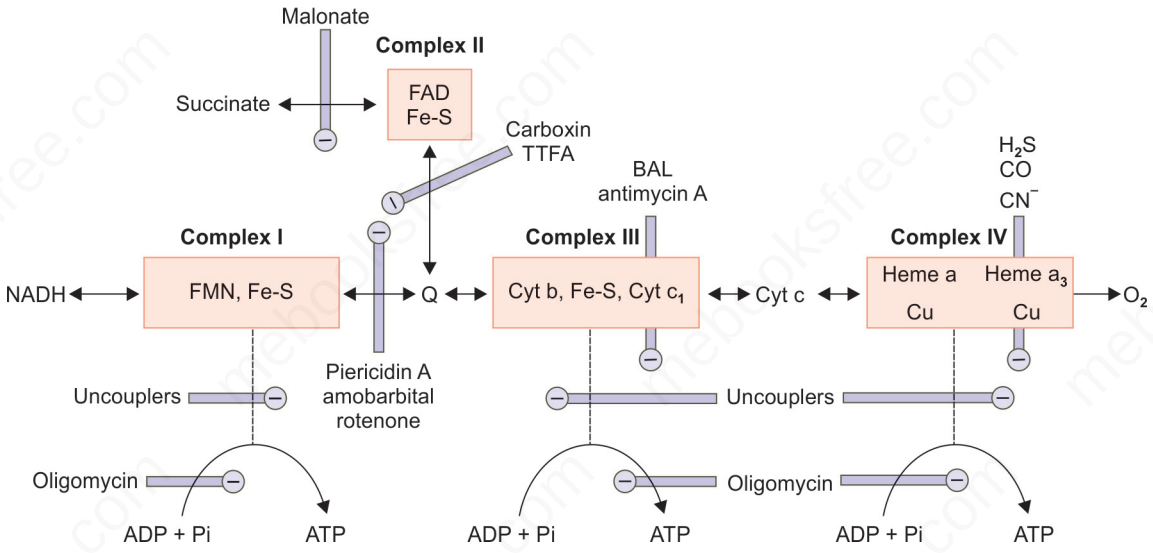


Fig. 17.10: Inhibitors of ETC

F1 Subcomplex

- Hydrophilic in nature
- Projects into the mitochondrial matrix
- F1 is attached to F0 Subcomplex
- Made up 9 Subunits ($\alpha_3\beta_3\gamma\delta\varepsilon$)
- γ subunit in the form of a 'bent axle.'
- γ subunit is surrounded by 3α and 3β subunit alternatively
- The flow of protons through F0 causes, rotation of F0 Complex along with γ subunit of F1 complex to rotate
- This causes the production of ATP in the F1 complex
- β subunit of F1 Complex is called Catalytic Subunit.

ATP Synthase Complex as a rotor-stator molecular motor

- Because it has two functional unit.

A rotating subunit

- Consist of F0 Complex and γ Subunit of F1 Complex

A stationary subunit

- F1 Complex other than γ subunit.

Binding Change Mechanism

- The theory behind the ATP production in the β subunit of F1 Subcomplex
- Proposed by Paul Boyer

- States that re-entry of protons through F0 Subcomplex causes rotation of γ subunit which in turn causes conformational changes in the β subunits of F1 Subcomplex.

INHIBITORS OF ELECTRON TRANSPORT CHAIN

Divided into
- Inhibitors of Electron transfer
- Inhibitors of Oxidative Phosphorylation
- Uncouplers of Oxidative Phosphorylation
- Ionophores

Inhibitors of Electron Transfer

Between NADH and CoQ [At Complex I]
- An insecticide and a fish Poison Rotenone
- Amobarbital which is a barbiturate
- Piericidin A.

Inhibitor of Complex II
- TTFA (Tri enoyl TriFluoroAcetone) a Fe^{2+} Chelating agent
- Carboxin
- Malonate, a competitive inhibitor of Succinate Dehydrogenase.

Between Cyt b and Cyt c [At Complex III]
- *Antimycin A*
- *British Antilevisite [Dimercaprol]*

Inhibitor at Cytochrome c Oxidase [Complex IV]
- *CO*
- *Cyanide*
- *H_2S*
- *Sodium Azide.*

Inhibitors of Oxidative Phosphorylation

Atractyloside

By inhibiting the transporter of ADP into and ATP out of the mitochondrion.

Oligomycin an Antibiotic

Completely blocks[Q] oxidation and phosphorylation.

By blocking the flow of protons through F0 Complex of ATP Synthase.

Uncoupler of Oxidative Phosphorylation

Mechanism of Action—Disruption of Proton Gradient across the inner mitochondrial membrane
- 2,4 Dinitrophenol
- Dinitrocresol
- FCCP [Fluoro Carbonyl Cyanide Phenyl hydrazine]
- ? Aspirin in high dose.

Physiological Uncoupler
- Thermogenin [Uncoupling Protein 1] in Brown Adipose Tissue
- Thyroxine
- Long Chain Free Fatty Acid
- ? Unconjugated Bilirubin.

Ionophores
- Ionophores permit specificcations to penetrate membranes
- Dissipate Proton Gradient
- Valinomycin
- Gramicidin
- Nigercin.

P:O Ratio
- Represents the number of ATP molecules produced in terms of reducing equivalents oxidized
- No of inorganic Phosphates utilized for ATP production for every atom of oxygen consumed
- For NADH - 2.5
- For FADH2 -1.5.

High Energy Compounds[Q]
- Compounds which yield energy of atleast 7 kcal/m on hydrolysis
- Compounds whose free energy of hydrolysis more than that of ATP is called High energy phosphates
- Compounds whose free energy of hydrolysis less than that of ATP is called low energy phosphates.

Classification of High energy Phosphates
- Pyrophosphate, e.g. ATP
- Acyl Phosphate, e.g. 1,3 Bisphosphoglycerate
- EnolPhosphate, e.g. Phosphoenolpyruvate
- Thioester, e.g. Acetyl CoA, Succinyl CoA
- Phosphagen, e.g. Phosphocreatine, Phosphoarginine.

Remember
- All high energy compounds given yield energy higher than ATP.
- Most of the compound contain Phosphate group (hence also called High Energy Phosphates) except Acetyl CoA.

High Energy Compound	Free Energy kJ/mol	Free Energy Kcal/mol
Phosphoenolpyruvate	**-61.9**	**-14.8**
Carbamoyl phosphate	-51.4	-12.3

Contd...

Contd...

High Energy Compound	Free Energy kJ/mol	Free Energy Kcal/mol
1,3-Bisphosphoglycerate (to 3-phosphoglycerate)	-49.3	-11.8
Creatine phosphate	-43.1	-10.3
ATP→AMP + PPi	-32.2	-7.7
ATP → ADP + Pi	-30.5	-7.3
Glucose-1-phosphate	-20.9	-5.0
PPi	-19.2	-4.6
Fructose-6-phosphate	-15.9	-3.8
Glucose-6-phosphate	-13.8	-3.3
Glycerol-3-phosphate	-9.2	-2.2

Respiratory Quotient[Q]

Measurement of the ratio of the volume of carbon dioxide produced: volume of oxygen consumed in the oxidation of metabolic fuels.

Metabolic fuel	Energy Yield (kJ/g)	RQ (CO_2 Produced/ O_2 Consumed	Energy (kJ)/L O_2
Carbohydrate	16	1.00	20
Protein	17	0.81	20
Fat	37	0.71	20
Alcohol	29	0.66	20

REVIEW QUESTIONS

TCA Cycle

1. Which of the following is not an intermediate of TCA Cycle? (AIIMS 2014 May)

a. Acetyl CoA
b. Citrate
c. Succinyl CoA
d. Alpha Ketoglutarate

Ans. a. Acetyl CoA. *(Ref: Harper 30/e p161-167)*

Acetyl CoA and Oxaloacetate are the starting materials of TCA Cycle

2. Which of the following is true about Krebs Cycle? (JIPMER May 2015)

a. Pyruvate condenses with Oxaloacetate to form Citrate
b. Alpha ketoglutarate is a five Carbon compound
c. Oxidative Phosphorylation occurs in the cytoplasm only
d. Krebs cycle can operate in anaerobic condition

Ans. b. Alpha ketoglutarate is a five Carbon compound. *Ref: Harper 30/e p161-167*

- Acetyl CoA condenses with Oxaloacetate to form Citrate
- Oxidative phosphorylation occur in mitochondria by ETC
- Krebs cycle cannot operate in anaerobic condition.

3. Which of the following substance binds to CoA and condenses oxaloacetate to inhibit the TCA cycle: (AIIMS Nov 2010)

a. Malonate
b. Arsenite
c. Fluoroacetate
d. Fumarate

Ans. c. Fluoroacetate. *(Ref: Harper 30/e p161-167)*

- The poison fluoroacetate is found in some of plants, and their consumption can be fatal to grazing animals
- Some fluorinated compounds used as anticancer agents and industrial chemicals (including pesticides) are metabolized to fluoroacetate
- It is toxic because fluoroacetyl-CoA condenses with oxaloacetate to form fluorocitrate, which inhibits aconitase, causing citrate to accumulate.

4. First substrate of Krebs cycle is: (AIIMS May 2007)

a. Pyruvate
b. Glycine
c. HCI
d. Lipoprotein

Ans. a. Pyruvate. *(Ref: Harper 30/e p161-167)*

From these options best answer is Pyruvate.

5. Hyperammonemia inhibits TCA cycle by depleting: (PGI June 2009)

a. Oxaloacetate
b. Alpha ketoglutarate
c. Citrate
d. Succinyl CoA
e. Fumarate

Ans. b. Alpha ketoglutarate. *(Ref: Harper 30/e p168)*

Hyperammonemia, as occurs in advanced liver disease and a number of (rare) genetic diseases of amino acid metabolism, leads to loss of consciousness, coma and convulsions, and may be fatal. This is because of the withdrawal of α ketoglutarate to form glutamate (catalyzed by glutamate dehydrogenase) and then glutamine (catalyzed by glutamine synthetase), leading to reduced concentrations of all citric acid cycle intermediates, and hence reduced generation of ATP.

6. What is liberated when Citrate converted to Cis Aconitate? (NBE pattern Q)

a. H_2O
b. H_2
c. H_2O_2
d. CO_2

Ans. a. H_2O. *(Ref: Harper 30/e p163)*

- Citrate isomerized to Isocitrate by Aconitase Reversible reaction
- The reaction occurs in two steps: dehydration to cis-aconitate and rehydration to isocitrate.

7. False about reducing equivalents is: (NBE pattern Q)

a. They are NADH and NADPH
b. Only produced during primary metabolic pathway
c. Formed in TCA cycle
d. Formed in mitochondria

Ans. b. Only produced during primary metabolic pathway.

8. High energy phosphate is not produced in:

a. TCA cycle
b. Hexose Mono Phosphate pathway
c. Glycolysis
d. Beta Oxidation of Fatty Acid

Ans. b. HMP Pathway.

Pathways which do not synthesize ATP are

- HMP Pathway
- Rapaport Leubering Cycle
- Uronic acid pathway
- Alpha oxidation of Fatty acid
- Omega Oxidation of fatty acid

Electron Transport Chain

9. Transport of ADP in and ATP out of mitochondria is inhibited by: (Nov 2010)

a. Atractyloside
b. Oligomycin
c. Rotenone
d. Cyanide

Ans. a. Atractyloside. *(Ref: Harper 30/e p132, 133)*

- Atractyloside inhibits oxidative phosphorylation by inhibiting the transporter of ADP into and ATP out of the mitochondrion
- The antibiotic oligomycin completely blocks oxidation and phosphorylation by blocking the flow of protons through ATP synthase
- Barbiturates such as amobarbital, Rotenone and Piericidin A inhibit electron transport via Complex I
- Antimycin A and dimercaprol inhibit the respiratory chain at Complex III. The classic poisons H_2S, carbon monoxide, and cyanide inhibit Complex IV and can therefore totally arrest respiration. Malonate is a competitive inhibitor of Complex II.

10. The electron flow in cytochrome C oxidase can be blocked by: (AIIMS May 2006)

a. Rotenone
b. Antimycin-A
c. Cyanide
d. Actinomycin

Ans. c. Cyanide. *(Ref: Harper 30/e p132,133)*

Cytochrome Oxidase is inhibited by CO, HCN, H_2S and Na Azide.

11. Cytosolic Cytochrome C mediates: (AIIMS May 2006)

a. Apoptosis
b. Electron transport
c. Krebs cycle
d. Glycolysis

Ans. a. Apoptosis. *(Ref: Harper 30/e p127-130)*

Mitochondrial Cytochrome c is a mobile electron carrier in Electron Transport Complex. This also mediates Apoptosis.

12. High energy compounds is/are: (PGI May 2012)

a. ATP
b. Creatine Phosphate
c. Glucose 1 Phosphate
d. Glycerol 3 Phosphate
e. ADP

Ans. a. ATP, **b.** Creatine Phosphate.

(Ref: Harper 30/e p116)

High Energy Compound	Free Energy kJ/mol	Free Energy Kcal/mol
Phosphoenolpyruvate	**-61.9**	**-14.8**
Carbamoyl phosphate	-51.4	-12.3
1,3-Bisphosphoglycerate (to 3-phosphoglycerate)	-49.3	-11.8
Creatine phosphate	-43.1	-10.3
ATP→AMP + PPi	-32.2	-7.7
ATP → ADP + Pi	-30.5	-7.3
Glucose-1-phosphate	-20.9	-5.0
PPi	-19.2	-4.6
Fructose-6-phosphate	-15.9	-3.8
Glucose-6-phosphate	-13.8	-3.3
Glycerol-3-phosphate	-9.2	-2.2

13. In ETC, oxidative phosphorylation (ATP formation) is regulated by: (PGI MAY 2011)

a. NADH CoQ reductase
b. Cytochrome C oxidase
c. Glutathione reductase
d. Isocitrate dehydrogenase
e. CoQ Cytochrome C reductase

Ans. a, b, c, e. *(Ref: Harper 30/e p130, 133)*

Components of the Electron Transport Chain[Q] are contained in four large protein complexes:

- Complex I NADH CoQ Oxidoredutase
- Complex II CoQ Succinate Reductase
- Complex III CoQ Cytochrome c Oxidoreductase
- Complex IV Cytochrome C Oxidase.

14. Which component transfer four protons:

a. NADH-Q Oxidoreductase **(PGI Nov 2009)**
b. Cytochrome-C Oxidase
c. Cytochrome C–Q oxidoreductase
d. Isocitrate Dehydrogenase
e. Succinate Q Reductase

Ans. a. NADH-Q Oxidoreductase, **c.** Cytochrome C–Q oxidoreductase. *(Ref: Harper 29/e p130)*

- Complex I and III pumps 4 H^+
- Complex II pumps no protons
- Complex IV pumps 2 H^+.

15. The specialized mammalian tissue/organ in which fuel oxidation serves not to produce ATP but to generate heat is: (AI 2006)

a. Adrenal gland
b. Skeletal music
c. Brown adipose tissue
d. Heart

Ans. c. Brown adipose tissue.

Brown adipose tissue contains thermogenin, which is a physiological uncoupler of oxidative phosphorylation. This process is called Nonshivering Thermogenesis.

16. Electron transport chain involves all except: (Kerala 2011)

a. NADP
b. NAD
c. Coenzyme Q
d. FAD

Ans. a. NADP.

NADP is involved in reductive biosynthesis, not in ETC.

17. F0-F1 Complex, ATP synthase inhibitor is: (Kerala 2007)

a. Atractyloside
b. Oligomycin
c. Antimycin
d. Rotenone

Ans. b. Oligomycin. *(Ref: Harper 29/e p127)*

- Atractyloside inhibits oxidative phosphorylation by inhibiting the transporter of ADP into and ATP out of the mitochondrion
- The antibiotic oligomycin completely blocks oxidation and phosphorylation by blocking the flow of protons through ATP synthase.

18. Respiratory Quotient 0.7 is seen in: (NBE pattern Q)

a. Carbohydrates
b. Fat
c. Protein
d. Alcohol

Ans. b. Fat.

Respiratory Quotient

Measurement of the ratio of the volume of carbon dioxide produced: volume of oxygen consumed

(Respiratory Quotient, RQ) ***is an indication of the mixture of metabolic fuels being oxidized.***

Metabolic fuel	Energy Yield (kJ/g)	RQ (CO_2 produced/O_2 consumed	Energy (kJ)/L O_2
Carbohydrate	16	1.00	20
Protein	17	0.81	20
Fat	37	0.71	20
Alcohol	29	0.66	20

19. Phenobarbitone inhibits which complex of ETC: (NBE pattern Q)

a. Complex I
b. Complex II
c. Complex III
d. Complex IV

Ans. a. Complex I. *(Ref: Harper 30/e p133)*

Inhibitors of ETC at Complex I

- An insecticide and a fish Poison Rotenone
- Amobarbital which is a barbiturate
- Piericidin.

20. Dinitrophenol inhibits the electron transport chain by: (NBE Pattern Q)

a. Cytochrome b
b. Inhibit ATP synthesis and electron transport chain
c. Inhibits ATP synthesis but not electron transport chain
d. Inhibits electron transport chain but not ATP synthesis

Ans. c. Inhibit ATP synthesis and but not electron transport chain.

Dinitrophenol is an uncoupler of Oxidative Phosphorylation. So no ATP synthesis but electron transfer and oxidation of reducing equivalents takes place.

21. Mechanism of Cyanide poisoning: (NBE pattern Q)

a. Inhibition of Cytochrome Oxidase
b. Inhibition of Carbonic Anhydrase
c. Inhibition of Cytochrome c
d. Inhibition of ATP Synthase

Ans. a. Inhibition of Cytochrome Oxidase.
(Ref: Harper 30/e p133)

Inhibitors of Complex IV are CO, Cyanide, H2S, Sodium Azide.

22. Final acceptor of electrons in ETC is: (AIIMS 2014 May)

a. Cyt c
b. Oxygen
c. FADH2
d. CoQ

Ans. b. Oxygen. *(Ref: Harper 30/e p133)*

- Electrons are transferred in the ascending order of redox potential, the final oxygen electron acceptor is oxygen.

18 Free Radicals, Xenobiotics and Metabolism of Alcohol

Topics Included

- Definition and Generation of Free Radicals
- Measurement of Body Free Radical Burden
- Free Radical Scavenging System

FREE RADICALS

Definition

Free radical is molecule or molecular fragment that contains one or more unpaired electrons in its outer orbit and has an independent existence.

A free radical is designated by a superscript dot ($R^{\bullet}$).

Generation of Oxygen Free Radicals (OFR) or Reactive Oxygen Species (ROS)

Incomplete reduction of Oxygen

In the body oxidative reactions normally ensures that molecular oxygen is completely reduced to water. Normally, four electrons are transferred to molecular oxygen so that it is completely reduced to form a water molecule.

$$4e^- + O_2 \xrightarrow{4H^+} 2H_2O$$

Incomplete reduction of oxygen generates Oxygen free radicals or Reactive Oxygen Species. They are Superoxide, Hydrogen Peroxide and Hydroxy radical.

- **Superoxide Radical are produced when a single electron is transferred to oxygen. It is both an anion and free radical.**

  $\bullet O_2 + e^- \longrightarrow \bullet O_2^-$ **(Superoxide)**

- **Hydrogen Peroxide: The two electron reduction product of oxygen is hydrogen peroxide (H_2O_2)**

  $\bullet O_2 + e^- \xrightarrow{2H^+} H_2O_2$ **(Hydrogen peroxide)**

This reaction is called Dismutation reaction. This can occur spontaneously or as enzyme catalyzed reaction.

Remember

Hydrogen Peroxide is not a free radical as it does not have an unpaired electron, but it is a Reactive oxygen species.

- **Hydroxy Radical: *The three electron reduction product of Oxygen is* Hydroxy radical (OH•). This is the most powerful oxygen free radical.**

Free Radicals generated by Iron

The electron is transferred from a Ferrous ion to Hydrogen Peroxide. This reaction is called **Fenton reaction.**

$$H_2O_2 + Fe^{2+} \longrightarrow OH_2^- + OH^{\bullet} + Fe^{3+}$$

***Another iron catalyzed reaction for generation of hydroxyl radical is* Haber Weiss Reaction.**

$$O_2^- + H_2O_2 \xrightarrow{Fe^{2+}} O_2 + OH^{\bullet} + OH^-$$

Sequential univalent reduction of oxygen

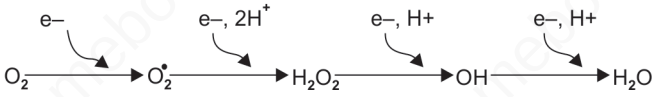


Fig. 18.1: Reactive oxygen species (ROS)

Points to remember

- Most powerful oxygen free radical is Hydroxyradical $(OH\bullet)^Q$
- Least powerful Reactive Oxygen species is Hydrogen peroxide (H_2O_2).
- Precursor of all reactive Oxygen species is Superoxide radical ($\bullet O2^-$)
- H_2O_2 is not a free radical but a ROS

Common sources of free radicals in the body

- Electron leakage in mitochondrial Electron Transport Chain.
- Normal Oxidation reduction reactions in the body
  - Xanthine Oxidase, Aldehyde Oxidase, dihydroorotate Dehydrogenase
  - Flavin Coenzymes in Peroxisomes generate H_2O_2.
  - L- Amino Acid Oxidase (Coenzyme-FMN)
  - D-Amino Acid Oxidase (Coenzyme-FAD)
- Respiratory Burst

Respiratory Burst

NADPH Oxidase in the inflammatory cells (neutrophils, eosnophils, monocytes and macrophages) produces superoxide anion by a process of respiratory burst during phagocytosis.

Steps of Respiratory Burst (See fig 18.2)

- The enzyme NADPH Oxidase catalyzes the formation of Superoxide radical from oxygen and NADPH
- By Dismutation Hydrogen Peroxide is generated
- Hydrogen Peroxide generate Hydroxy radical spontaneously
- Hydrogen peroxide generate Hypochlorous acid by the action of enzyme Myeloperoxidase **exclusively present in the neutrophil granules**
- They mediate killing of bacteria. This is called respiratory burst.

Damages produced by free radicals

- Free radicals are highly reactive molecular species with an unpaired electron
- They persist for only a very short time (of the order of **10^{-9} to 10^{-12} sec**) before they collide with another molecule and either abstract or donate an electron in order to achieve stability
- In so doing, they generate a new radical from the molecule with which they collided
- The main way in which a free radical can be quenched, so terminating this chain reaction, is if two radicals react together, when the unpaired electrons can become paired in one or other of the parent molecules
- They cause damage to **nucleic acids, proteins, and lipids in cell membranes and plasma lipoproteins**
- This can cause cancer, atherosclerosis and coronary artery disease, and autoimmune diseases.

Lipid Peroxidation

Lipids are most susceptible to damaging effects of free radicals. PUFA present in cell membrane and plasma lipoproteins are especially prone to damage.

Radical damage to unsaturated fatty acids in cell membranes and plasma lipoproteins leads to the formation of lipid peroxides, then highly reactive dialdehydes[Q] that can chemically modify proteins and nucleic acid bases.

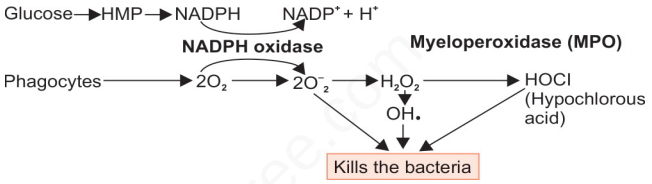


Fig. 18.2: Respiratory burst

MEASUREMENT OF BODY FREE RADICAL BURDEN

The total body radical burden can be estimated by measuring the products of lipid peroxidation.

- **FOX (Ferrous Oxidation in Xylenol) Assay**
  - Lipid peroxides can be measured by the ferrous oxidation in xylenol orange (FOX) assay. Under acidic conditions, they oxidize Fe^{2+} to Fe^{3+}, which forms a chromophore with xylenol orange.
- **Estimation of Dialdehydes**
  - The dialdehydes formed from lipid peroxides can be measured by reaction with thiobarbituric acid, when they form a red fluorescent adduct. The results of this assay are generally reported as total **t**hio**b**arbituric **a**cid **r**eactive **s**ubstances, **TBARS**.
- **Measurement of Pentane and Methane in Exhaled air.**
  - Peroxidation of ω-6 polyunsaturated fatty acids leads to the formation of pentane, and of-ω3 polyunsaturated fatty acids to ethane, both of which can be measured in exhaled air.

FREE RADICAL SCAVENGING SYSTEM

Antioxidants fall into two classes:

- Preventive antioxidants, which reduce the rate of chain initiation
  - They are Glutathione Peroxidase, Catalase
- Chain-breaking antioxidants, which interfere with chain propagation
  - They are Superoxide Dismutase, Uric Acid, Vitamin E.

Preventive Antioxidants

Glutathione Peroxidase

- Eliminates hydrogen peroxide and organic hydroperoxides by reaction with reduced Glutathione (GSH)

- Reduced Glutathione is converted to oxidized glutathione (GSSG)
- Glutathione Reductase convert oxidized Glutathione back to reduced Glutathione using the reducing equivalent NADPH
- Glutathione Peroxidase is a selenium containing enzyme.

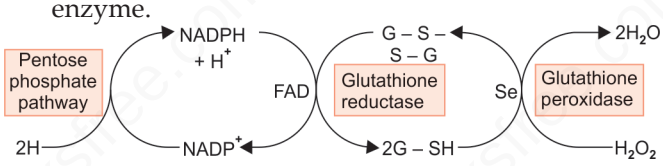


Fig. 18.3: Free radical scavenging by glutathione peroxidase

Catalase

- It is a hemoprotein with four heme groups. Causes decomposition of peroxides to yield water and oxygen
- Highest concentration of Catalase is present in Peroxisomes.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Remember
H_2O_2 is not free radical but can generate free radical.

Chain Breaking Antioxidant

Superoxide Dismutase

It is the only enzyme that takes a free radical as its substrate, hence a scavenger. Two isoenzymes are there for Super Oxide Dismutase (SOD).

Cytosolic SOD is Copper dependent and Mitochondrial SOD is Manganese dependent[Q].

$$\cdot O_2^- + \cdot O_2^- + 2H+ \xrightarrow{\text{Superoxide Dismutase}} H_2O_2 + 2H+$$

Other Nonenzymic Antioxidants

Vitamin E (α-Tocopherol)

- Most potent chain breaking antioxidant
- Being lipophilic it acts on biological membranes
- It terminates lipid peroxidation.

Other antioxidants are:

- **β Carotene and Ubiquinone** are lipid soluble radical-trapping antioxidants in membranes and plasma lipoproteins
- **Ascorbate, uric acid** and a variety of polyphenols derived from plant foods act as water-soluble radical trapping antioxidants, forming relatively stable radicals that persist long enough to undergo reaction to nonradical products.

Antioxidants can be prooxidants called as antioxidant paradox

- **Ascorbate**, can also be a source of superoxide radicals by reaction with oxygen, and hydroxyl radicals by reaction with Cu^{2+} ions
- β-carotene is indeed a radical-trapping antioxidant under conditions of low partial pressure of oxygen, as in most tissues, at high partial pressures of oxygen (as in the lungs) and especially in high concentrations, β-carotene is an autocatalytic prooxidant
- Increased mortality among those taking supplements of **Vitamin E.**

XENOBIOTICS

A **xenobiotic** (Gk xenos 'stranger') is a compound that is foreign to the body.

Metabolism of xenobiotics in two phases

- **In phase 1 reactions**, xenobiotics are generally converted to more **polar, hydroxylated derivatives**
- **In phase 2 reactions**, these derivatives are conjugated with molecules such as glucuronic acid, sulfate, or glutathione. This renders them even more water-soluble, and they are eventually excreted in the urine or bile.

Phase 1 Reactions

- **Hydroxylation,** catalyzed mainly by members of a class of enzymes referred to as **monooxygenases** or **cytochrome** P450s
- Deamination
- Dehalogenation
- Desulfuration
- Epoxidation
- Peroxygenation
- Reduction.

Biotransformation to Toxic Compounds

In certain cases, phase 1 metabolic reactions convert xenobiotics from **inactive** to **biologically active** compounds. In these instances, the original xenobiotics are referred to as **'prodrugs'** or **'procarcinogens.'**

Example:

- Vinyl Chloride to Vinyl Chloride Epoxide which can bind to DNA and RNA
- Mercury methylated, making them neurotoxic

- Methanol to formic acid
- Benzopyrene converted to its epoxide by epoxidation.

Cytochrome P450

The main reaction involved in phase 1 metabolism is **hydroxylation**, catalyzed by a family of enzymes known as **monooxygenases** or **'mixed-function oxidases**.'

- There are at least 57 cytochrome P450 genes in the human genome
- Cytochrome P450 is a heme enzyme
- They exhibit an absorption peak at 450 nm
- Approximately 50% of the common drugs that humans ingest are metabolized by isoforms of cytochrome P450
- They also act on steroid hormones, carcinogens, and pollutants
- In addition to their role in metabolism of xenobiotics, cytochromes P450 are important in the metabolism of a number of physiological compounds—for example, the synthesis of steroid hormones and the conversion of vitamin D to its active metabolite, calcitriol
- NADPH is required to reduce cytochrome P450
- Lipids which are components of CytP450 is Phosphatidyl Choline.

Sites where CytP450 is present

- In mammals, cytochromes P450 are present in highest amount in **liver cells** and enterocytes but are probably present in all tissues
- In liver and most other tissues, they are present mainly in the **membranes of the smooth endoplasmic reticulum (microsomal fraction)**
- In the **adrenal gland**, they are found in **mitochondria** as well as in the endoplasmic reticulum
- In the adrenal gland are involved in cholesterol and steroid hormone biosynthesis.
- The mitochondrial cytochrome P450 system differs from the microsomal system in that it uses an NADPH-linked flavoprotein, **adrenodoxin reductase**, and a nonheme iron-sulfur protein, **adrenodoxin**.

Properties of Human Cytochromes P450

- Involved in phase I of the metabolism of a large number of xenobiotics, including perhaps 50% of the clinically used drugs;
- Involved in the metabolism of many endogenous compounds (e.g., steroids)
- All are hemoproteins
- Often exhibit broad substrate specificity, thus acting on many compounds; consequently, different P450s may catalyze formation of the same product

Contd...

Contd...

- Basically they catalyze reactions involving introduction of one atom of oxygen into the substrate and one into water
- Their hydroxylated **products are more water-soluble than** their generally lipophilic substrates, facilitating excretion
- Liver contains highest amounts, but found in most if not all tissues, including small intestine, brain, and lung
- Located in the smooth endoplasmic reticulum or in mitochondria (steroidogenic hormones)
- In some cases, their products are mutagenic or carcinogenic.
- Many have a molecular mass of about 55 kDa
- Many are **inducible**, resulting in one cause of drug interactions
- Some exhibit genetic polymorphisms, which can result in atypical drug metabolism
- Their activities may be altered in diseased tissues (e.g. cirrhosis), affecting drug metabolism.

Phase 2 Reactions

Most abundant Phase 2 reaction is Conjugation

Glucuronidation

- The glucuronidation of bilirubin by UDP-glucuronic acid
- Glucuronidation is probably the most frequent conjugation reaction.

Sulfation

- The sulfate donor in these and other biologic sulfation reactions) is **adenosine 3'-phosphate-5'-phosphosulfate (PAPS)**, this compound is called 'active sulfate.'
- For example, sulfation of steroids, glycosaminoglycans, glycolipids, and glycoproteins.

Conjugation with Glutathione

- Glutathione (γ-glutamylcysteinylglycine) is a **tripeptide** consisting of glutamic acid, cysteine, and glycine
- Glutathione is commonly abbreviated GSH (because of the sulfhydryl group of its cysteine, which is the business part of the molecule)
- The enzymes catalyzing these reactions are called glutathione S-transferases and are present in high amounts in liver cytosol and in lower amounts in other tissues.

Acetylation

- **Acetyl-CoA** (active acetate) is the acetyl donor. These reactions are catalyzed by **acetyltransferases.**

Methylation

- A few xenobiotics are subject to methylation by methyltransferases, employing S-adenosylmethionine as the methyl donor.

Conjugation with Glycine

Benzoic acid conjugated with Glycine to form Hippuric Acid (Benzoyl Glycine)

Conjugation with Glutamine

Phenyl Acetic Acid is conjugated with Glutamine to form Phenyl Acetyl Glutamine.

METABOLISM OF ALCOHOL

Topics included

- Metabolism of Alcohol
- Metabolic alterations following ingestion of alcohols

Metabolism of Alcohol

- NAD+ Dependent **Cytoplasmic** Alcohol Dehydrogenase oxidises Ethanol to Acetaldehyde.
- Acetaldehyde is further oxidized to Acetate by **mitochondrial** NAD+ dependent Aldehyde Dehydrogenase. Activity of Alcohol Dehydrogenase is more than Aldehyde dehydrogenase.
- So, Acetaldehyde accumulates in the liver
- **Aldehyde** is toxic and causes cell death.

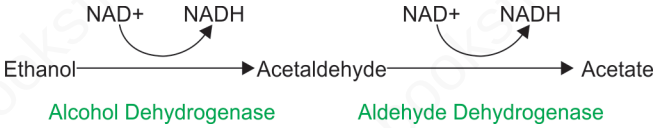


Fig. 18.4: Metabolism of alcohol

Metabolic Changes Following Ingestion of Alcohol

High concentration of NADH leads to high NADH/NAD+ ratio. This is the basic cause of all metabolic alteration in alcoholism.

- Favor conversion of Pyruvate to Lactate (This leads to **Lactic acidosis**)
- Deficiecy of Pyruvate leads to deficiency of Oxaloacetate. This leads to **decrease in Gluconeogenesis** which causes hypoglycemia.
- Decreased availability of NAD+ and decreased Oxaloacetate lead to **decreased TCA Cycle** leads to accumulation of Acetyl CoA
- Accumulation of Acetyl CoA leads to **Ketogenesis and Lipogenesis** and **Fatty Liver**.
- Lactic Acidosis leads to decreased Uric Acid excretion and hence **gout**.

Protective Mechanism of Microsomal Ethanol Oxidizing System (MEOS)

- Some metabolism of ethanol takes place via a cytochrome P450-dependent microsomal ethanol oxidizing system (MEOS) involving **NADPH and O_2**.
- So NADH/NAD+ ratio is not altered hence account for metabolic tolerance in chronic alcoholics.
- This system is inducible hence increases in activity in **chronic alcoholism.**
- In some Asian populations and Native Americans, alcohol consumption results in increased adverse reactions to acetaldehyde owing to a genetic defect of mitochondrial aldehyde dehydrogenase.

REVIEW QUESTIONS

Free Radicals

1. Free radical with highest activity:

a. O_2^-
b. OH•
c. Hypochlorite
d. Peroxynitrite

Ans. b. OH•

Points to Remember

- Most powerful oxygen free radical is **Hydroxyradical (OH•)**[Q]
- Least powerful Reactive Oxygen species is **Hydrogen peroxide (H_2O_2).**
- Precursor of all reactive Oxygen species is **Superoxide radical (O_2^-)**

2. Most powerful chain breaking antioxidant:

a. Glutathione peroxidase
b. Alpha tocopherol
c. Superoxide dismutase
d. Vitamin C

Ans. b. Alpha tocopherol

3. Enzyme which catalyze the reaction H_2O_2 give $H_2O + O_2$:

a. Catalase
b. Glutathione reductase
c. Glutathione peroxidase
d. Glutathione s transferase

Ans. a. Catalase

Catalase

It is a hemoprotein with four hemegroups. Causes decomposition of peroxides to yield water and oxygen. Highest concentration of Catalase is present in Peroxisomes.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Remember

H_2O_2 is not free radical but can generate free radical.

4. Which of the following is not a free radical?

a. Hydroxyl radical
b. Hydrogen Peroxide
c. Superoxide
d. $O_2^{\bullet}$

Ans. b. Hydrogen Peroxide.

It is a reactive oxygen species, not a free radical.

Alcohol Metabolism

5. Toxicity of Ethanol is due to: (Jipmer 2012)

a. Increased NADH/NAD+ ratio
b. Decreased lactate/Pyruvate ratio
c. Inhibition of Gluconeogenesis
d. Stimulation of fatty acid oxidation

Ans. a. Increased NADH/NAD+ ratio

High concentration of NADH leads to high NADH/NAD+ ratio. This is the basic cause of all metabolic alteration in alcoholism.

6. Best explained pathogenesis of fatty liver in alcoholic liver disease: (Jipmer 2013)

a. Increased hydrolysis of fat from adipocytes
b. Decreased synthesis of fatty acids
c. Decreased [NADH]/[NAD+] ratio
d. Impaired beta oxidation of fatty acids

Ans. d. Impaired beta oxidation of fatty acids

Impaired beta oxidation leads to increased TAG in the liver, which leads to fatty liver.

6

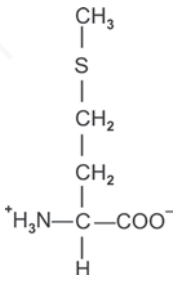
Section I

Image-Based Questions

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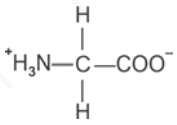
IMAGE-BASED QUESTIONS

1. Identify the amino acid given in the diagram.



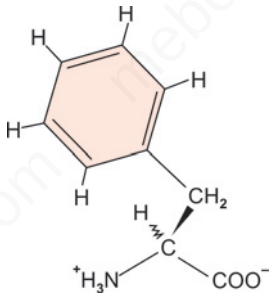
a. Cysteine
b. Cystine
c. Methionine
d. Homocysteine

2. The best description of the given amino acid is:



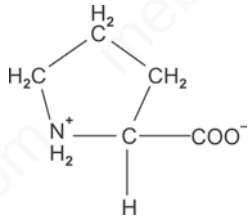
a. Simple, Polar, Nonessential
b. Branched chain, Polar, Essential
c. Branched chain, nonpolar, Nonessential
d. Optically active

3. The amino acid given in the picture is hydroxylated to form which amino acid?



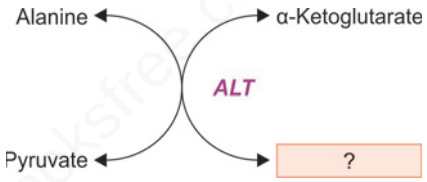
a. Serine
b. Threonine
c. Tyrosine
d. Tryptophan

4. The amino acid given in the diagram can be is best suited to which of the following description?



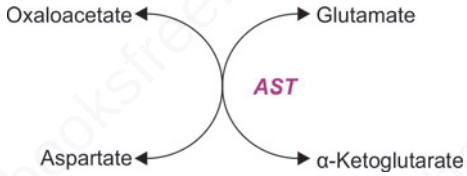
a. Answer Ninhydrin test with purple coloured complex
b. Alpha amino acid with polar side chain
c. Nonpolar imino acid
d. Aromatic essential amino acid

5. In the reaction, what is product formed?



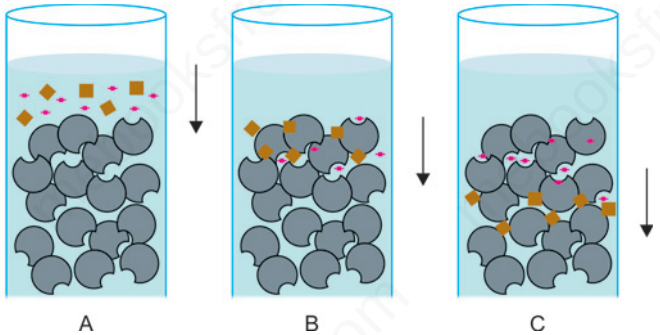
a. Glutamate
b. Glutamine
c. Oxaloacetate
d. Aspartate

6. What is the vitamin that act as the coenzyme of the given reaction?



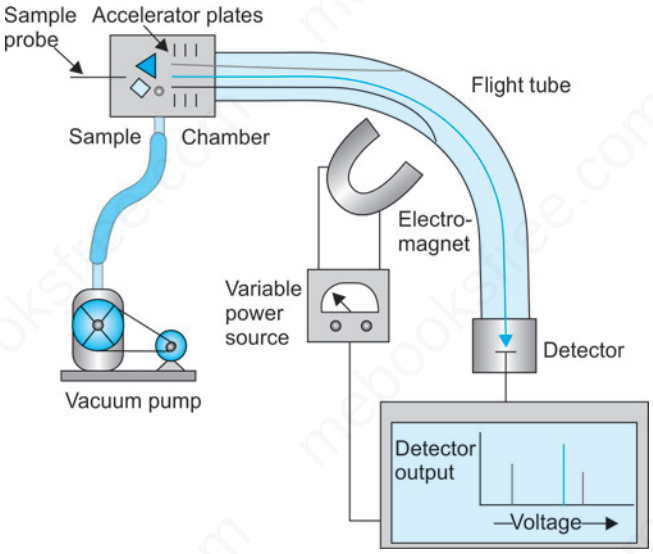
a. Vitamin B1
b. Vitamin B2
c. Vitamin B5
d. Vitamin B6

7. Identify the separatory technique of protein given in the picture?



a. Size Exclusion Chromatography
b. Affinity Chromatography
c. Ion Exchange Chromatography
d. High Pressure Liquid Chromatography

8. **All the following description about the given technique is true *except*:**

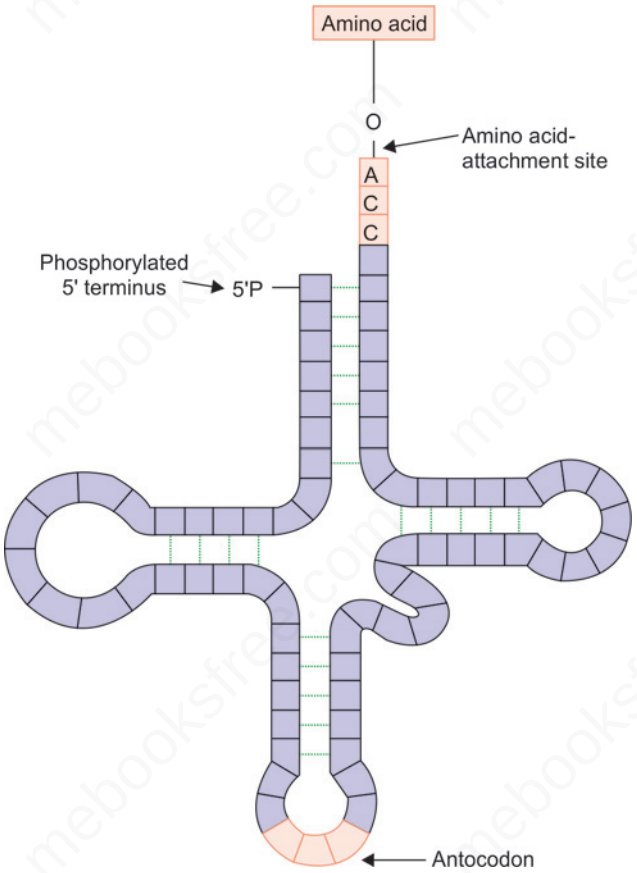


a. Help to sequence the polypeptide
b. Gold standard screening method of metabolic disorders
c. Differentiate biological compounds based on mass to charge ratio
d. Can also be used to study the secondary and tertiary structures of Proteins.

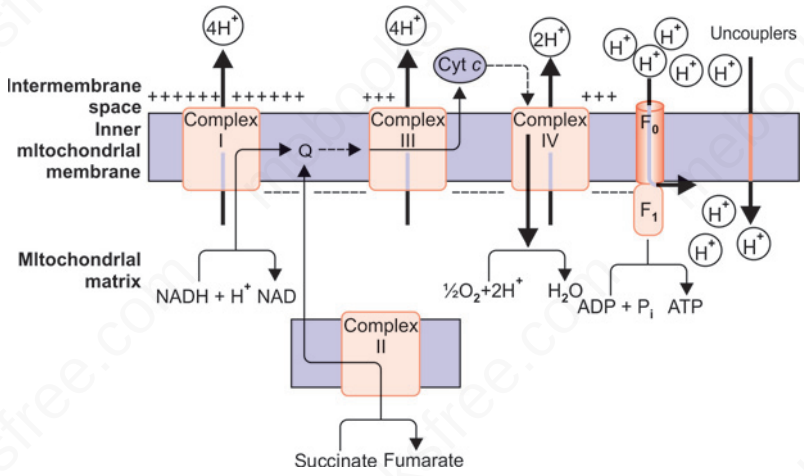
9. **What is the name of the complex that pumps 2 protons to the intermembrane space of mitochondria in the given Electron Transport Chain?**

a. NADH CoQ Oxidoreductase
b. Succinate CoQ Oxidoreductase
c. Cytochrome C Oxidase
d. Q Cyt C Oxidoreductase

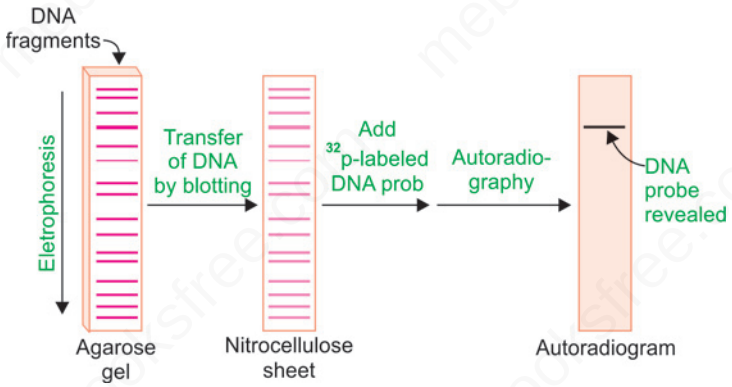
10. **All the following are true about the given structure *except*:**



a. Intrastrand base pairing is seen
b. This structure can be translated to a protein
c. 3′ end has CCA base sequence
d. Pseudouridine is an unusual amino acid present in this.



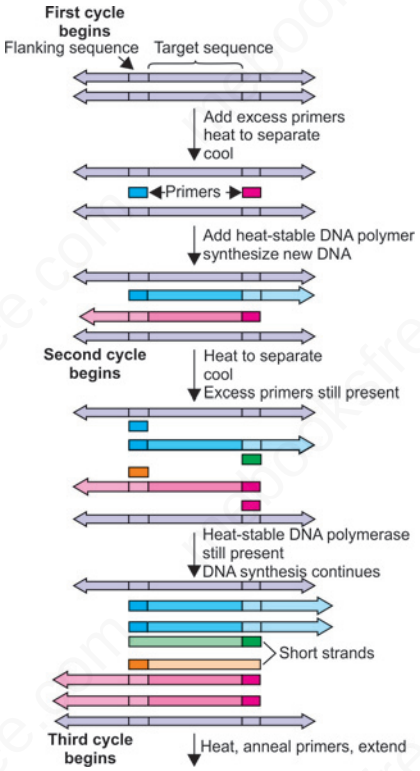
11. The given molecular biology technique can detect which of the following?



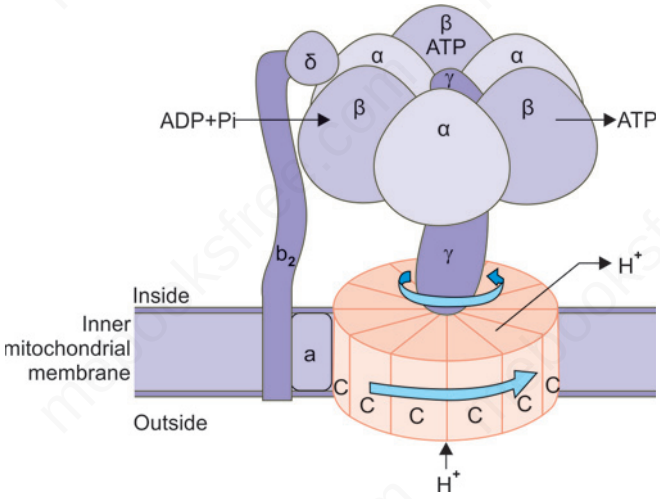
a. DNA
b. RNA
c. Protein
d. DNA Protein Interaction

12. All the following about the given techniques are true *except*:

a. Invented by Dr Karry B Mullis
b. Can amplify DNA and RNA
c. This is an *in vivo* technique
d. Exponential amplification is possible

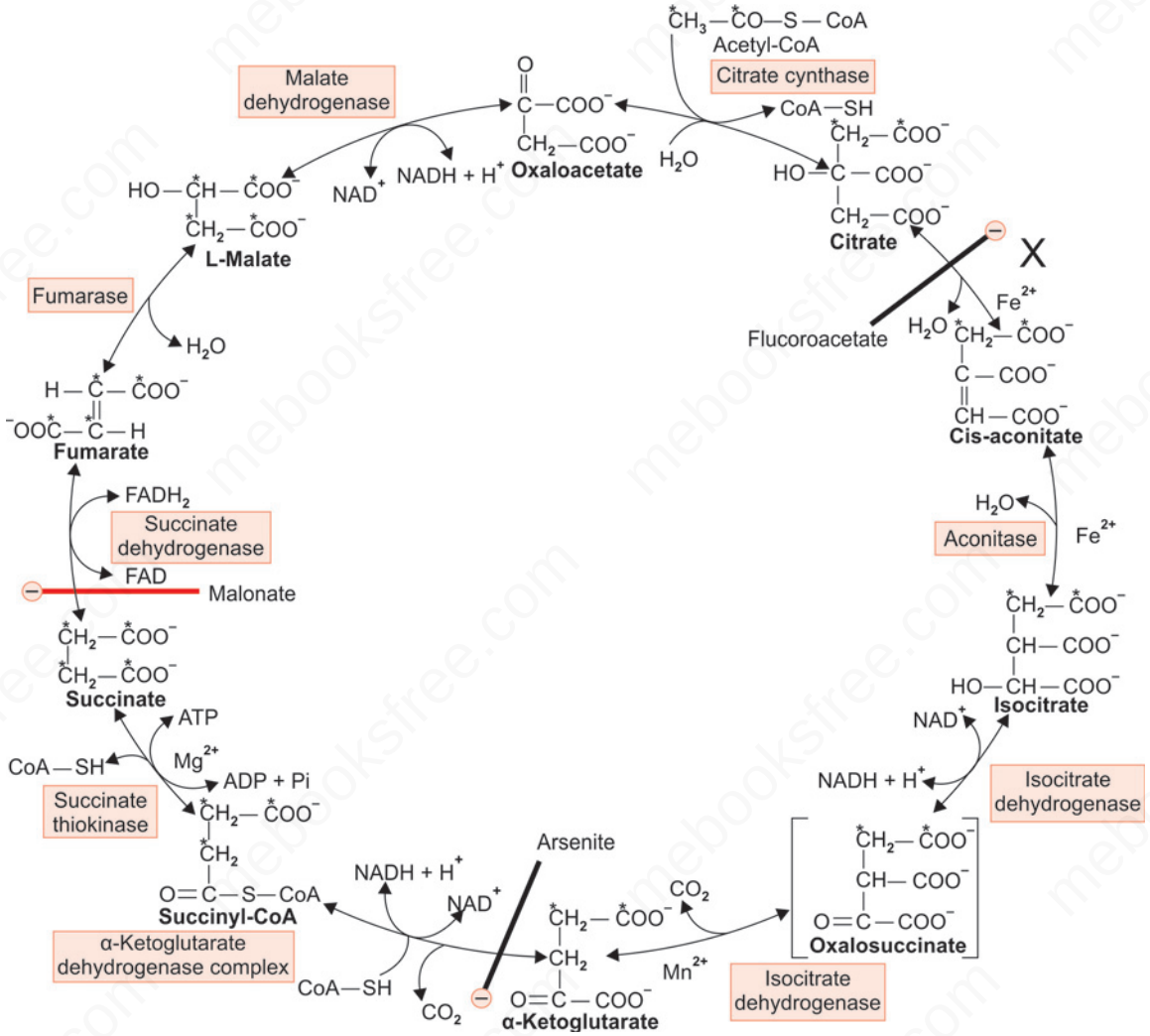


13. At which subunit ATP is formed in the given complex:

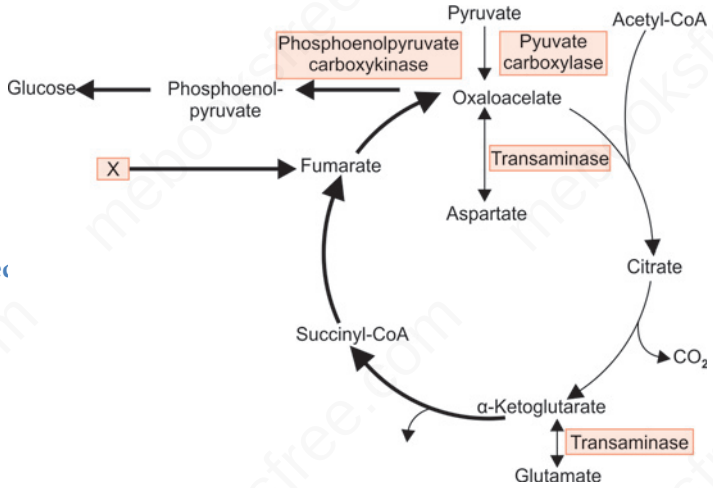


a. Fo Subcomplex
b. γ subunit of F1
c. C disc of Fo subcomplex
d. β subunit of F1 subcomplex

14. Identify the enzyme marked X:



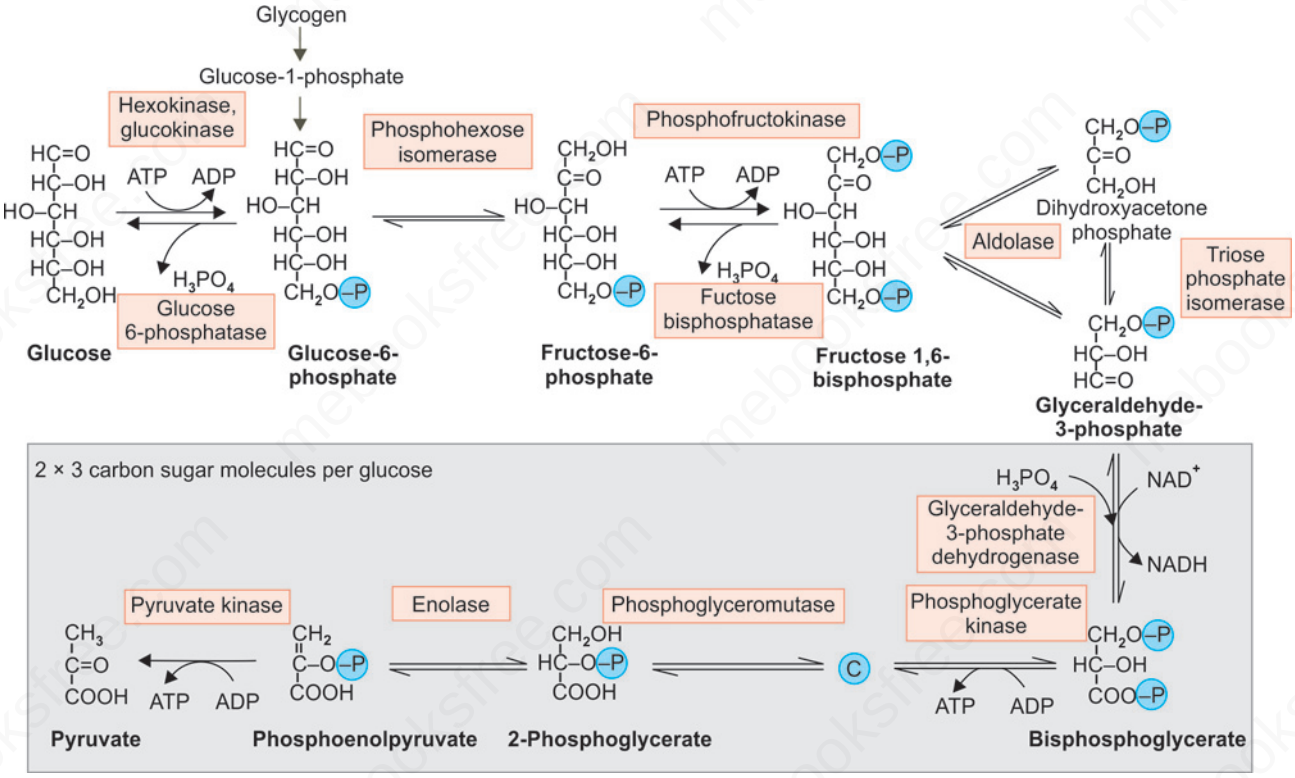
a. Citrate Isomerase
b. Aconitase
c. Citrate Cis-Trans isomerase
d. Isocitrate Synthase



15. Which of the following is the amino acid marke X, that enter at the level of Fumarate?

a. Alanine
b. Tryptophan
c. Tyrosine
d. Histidine

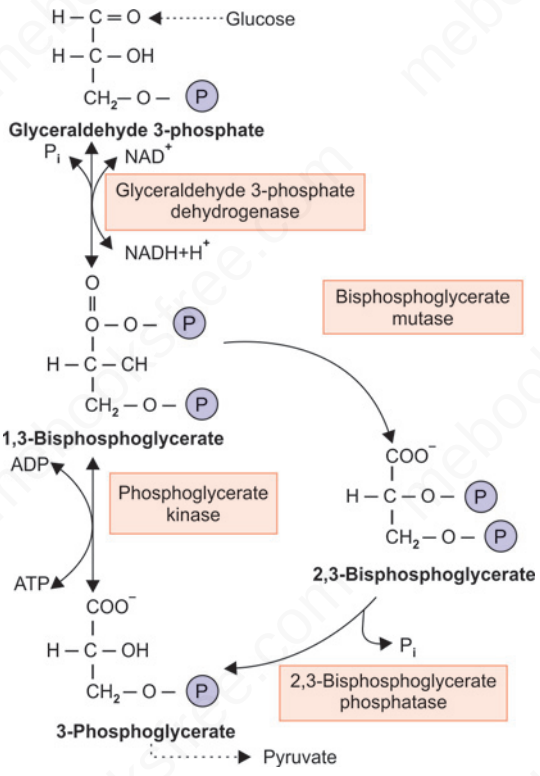
16. Identify the intermediate in the given pathway marked C?



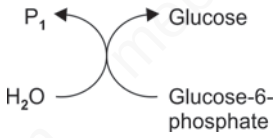
a. 1 Phosphoglycerate
b. 2, 3 Phosphoglycerate
c. 1, 3 Diphosphoglycerate
d. 3 Phosphoglycerate

17. All the following about this pathway are true *except*:

a. Takes place in RBC
b. This is the major fate of glucose in RBC
c. No net ATP is produced by this pathway
d. Substrate level phosphorylation step is bypassed

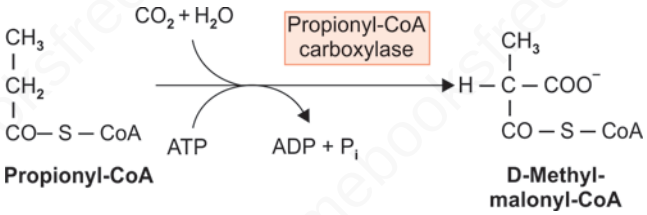


18. The enzyme catalyzing this pathway is absent in:



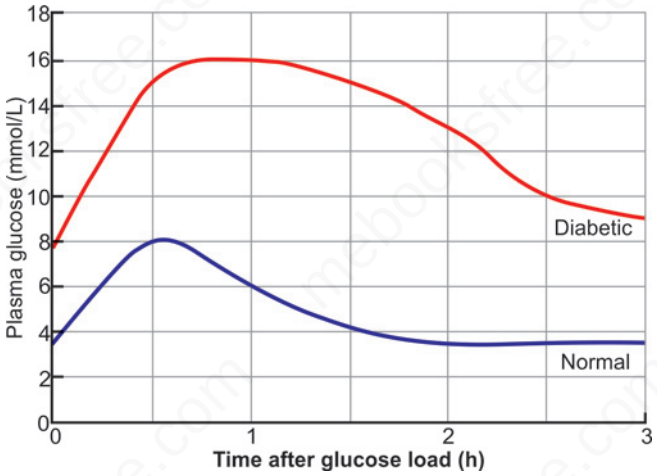
a. Liver
b. Muscle
c. GIT
d. Kidney

19. What is the coenzyme of this reaction?



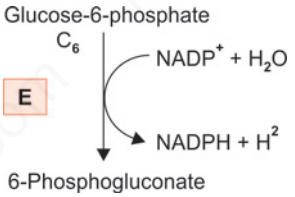
a. Vitamin B12
b. Biotin
c. Thiamine
d. Vitamin B6

20. What is the indication for doing this test in laboratory?



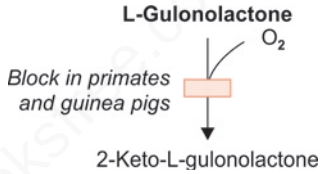
a. Overt Diabetes mellitus
b. Diabetic nephropathy
c. Symptoms of Diabetes mellitus, but Blood glucose values are inconclusive
d. Follow-up of Diabetes mellitus

21. Which of the following is NOT true regarding this enzyme, marked E?



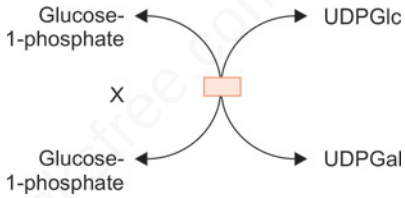
a. Rate limiting step of HMP Pathway
b. Nonoxidative phase of HMP pathway
c. Most common enzyme deficiency in human beings
d. Deficiency of this enzyme causes Methhemoglobinemia and Hemolytic anemia

22. Identify the enzyme that catalyse this reaction, that is absent in humans and higher primates?



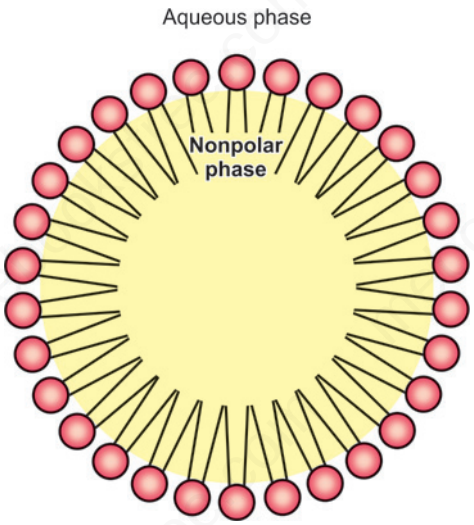
a. L-Gulanolactone Oxygenase
b. L-Gulanolactone Oxidase
c. L-Gulanolactone Hydroxylase
d. L-Gulanolactone Dehydrogenase

23. Which is true regarding the disorder caused by the deficiency of enzyme marked X?



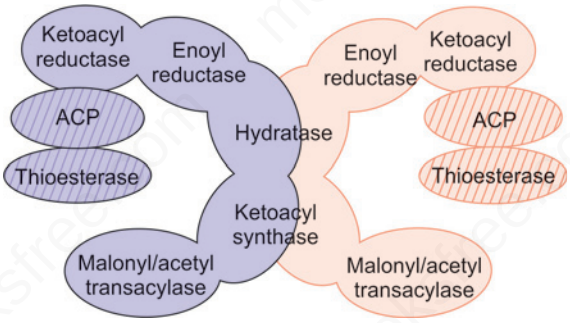
a. Cataract is the only clinical feature
b. Presents after 1 year of age
c. Benedict's test and Glucose oxidase test positive
d. They are at increased risk of neonatal sepsis by *E coli*.

24. Identify the given diagram



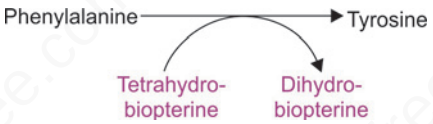
a. Liposome
b. Aquasome
c. Micelle
d. Lipid Bilayer

25. In which of the following pathway the given multienzyme complex takes part?



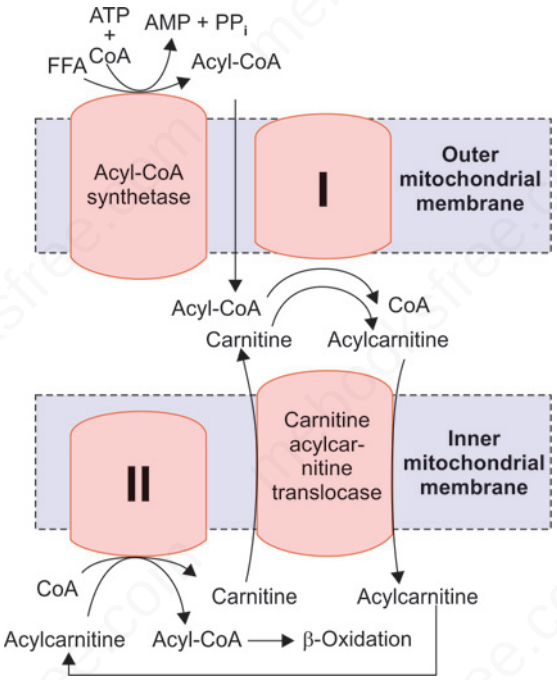
a. Alpha oxidation of fatty acid
b. De novo fatty acid synthesis
c. Beta oxidation of fatty acid
d. Omega oxidation of fatty acid

26. The enzyme catalyzing this reaction belongs to which class of enzyme?



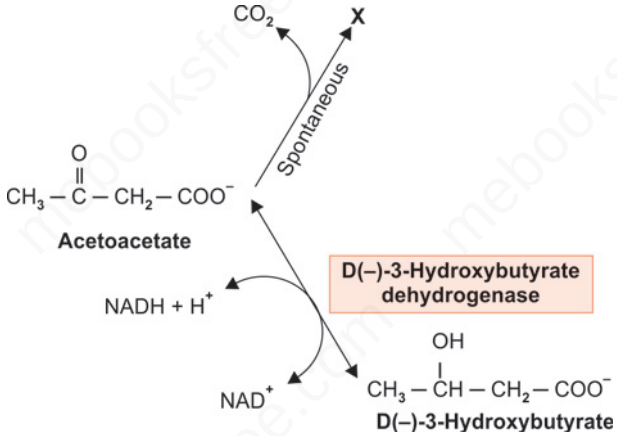
a. Monooxygenase
b. Diooxygenase
c. Oxidase
d. Dehydrogenase

27. The enzymes marked I and II in the figure are respectively:



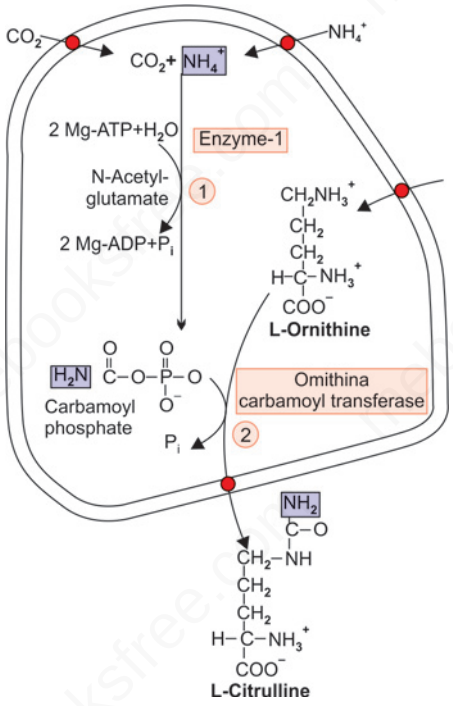
a. Carnitine Palmitoyl Transferase I and Carnitine Palmitoyl Transferase II
b. Carnitine Palmitoyl Transferase II and Carnitine Palmitoyl Transferase I
c. Carnitine Acyl Carnitine Translocase I and Carnitine Palmitoyl Transferase I
d. Carnitine Palmitoyl Transferase II and Carnitine Acyl Carnitine Translocase II

28. Identify the product X formed by the reaction given in the figure?



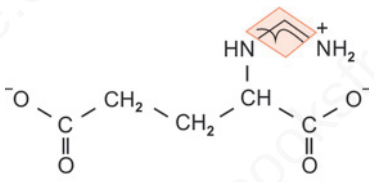
a. Beta Hydroxy Butyrate
b. Acetyl CoA
c. Acetate
d. Acetone

29. All are true about reaction catalysed by enzyme -1 *except*:



a. Rate limiting enzyme of the pathway
b. This enzyme defect is the most common cause of Hyperammonemia
c. Biotin independent Carboxylation
d. Takes place in the mitochondria.

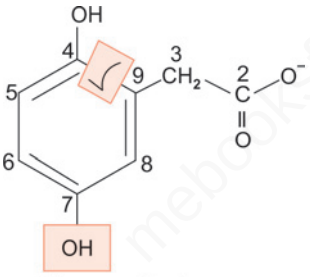
30. This compound is excreted in urine in which vitamin deficiency?



N-Formiminoglutamate (Figlu)

a. Folic acid deficiency
b. Cobalmin deficiency
c. Thiamine deficiency
d. Biotin deficiency

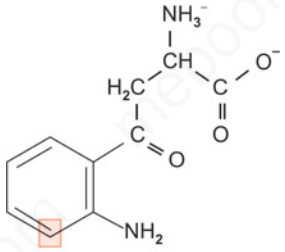
31. The false statement regarding the given compound is:



Homogentisate

a. Excreted in urine in Alkaptonuria
b. Gives Benedict's test positive
c. Polymerises to benzoquinone acetate
d. Strong oxidizing agent

32. The compound in the figure is an intermediate in the metabolism of which amino acid?



L-Kynurenine

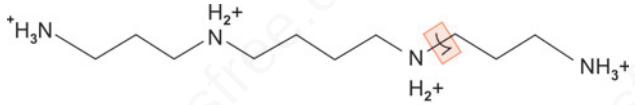
a. Tyrosine
b. Histidine
c. Tryptophan
d. Phenylalanine

33. The vitamin that acts as a coenzyme of the given reaction is

Serine + Homocysteine → Cystathionine + H_2O

a. Vitamin B2
b. Vitamin B3
c. Vitamin B5
d. Vitamin B6

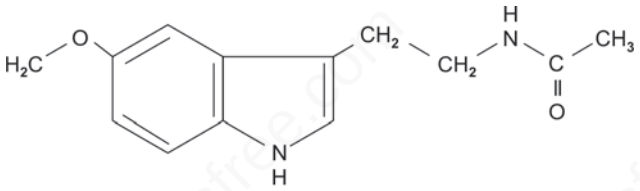
34. The rate limiting enzyme in the synthesis of this compound is



Spermine

a. Spermine Synthase
b. SAM Decarboxylase
c. Ornithine Decarboxylase
d. Ornithine transcarbamoylase

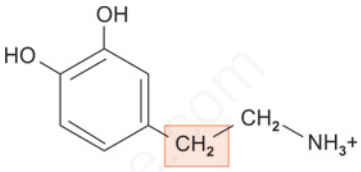
35. The functions of the given compound is:



Melatonin

a. Biological rhythm
b. Vasoconstriction
c. Mood Depression
d. Mood elevation

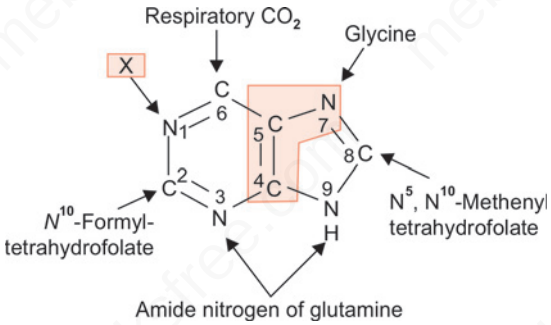
36. This compound is derived from which amino acid?



Dopamine

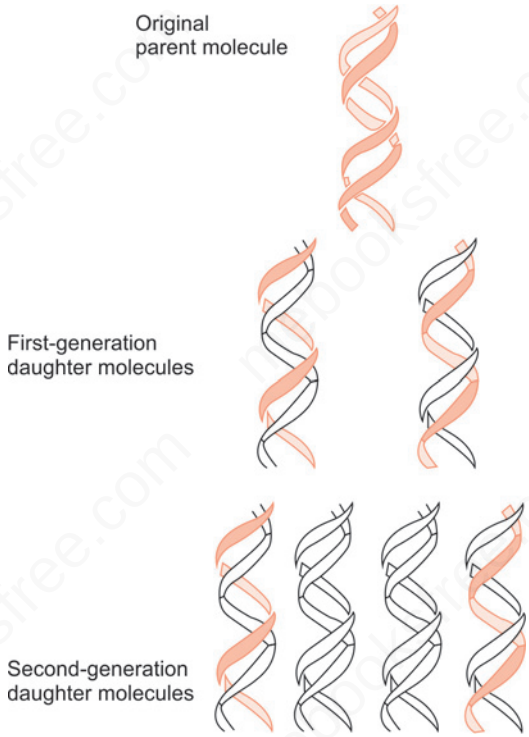
a. Tyrosine
b. Histidine
c. Tryptophan
d. Leucine

37. The compound marked X, that contribute to the Nitrogen in the given ring structure is



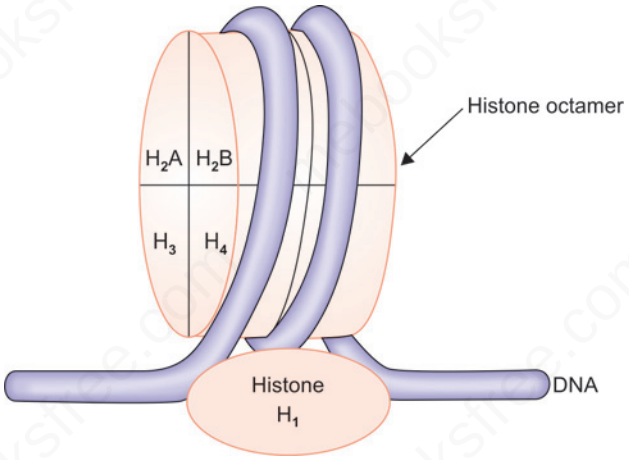
a. Asparagine
b. Aspartate
c. Glutamate
d. Serine

38. All the following statement about the given diagrams are true *except*?



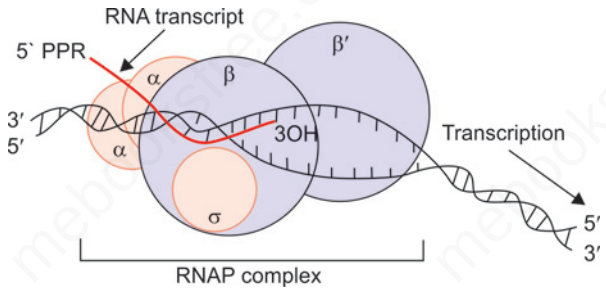
a. Half of the parent strand is conserved in the daughter DNA
b. Proved by Meselson and Stahl
c. The genetic information is passed from parent to progeny
d. This takes place in the M phase of Cell cycle

39. Which of the following is not true about the complex ?



a. Have beads on string appearance
b. DNA complexed with histone octamer
c. DNA is wound in right handed direction
d. Has 146 bp in the DNA helix that wound on the histone octamer

40. The complex in the diagram specifically binds to binds to which regions of DNA?



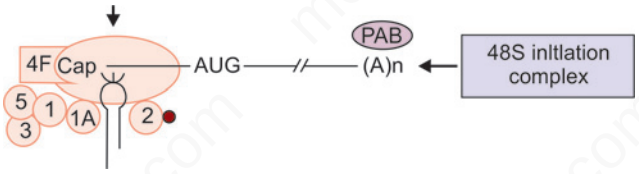
a. Enhancers
b. Promotors
c. Introns
d. Exons

41. The cracking of the code in the diagram is done by:

First nucleotide	Second Nucleotide				Third nucleotide
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Term	Term[b]	A
	Leu	Ser	Term	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile[a]	Thr	Lys	Arg[b]	A
	Met	Thr	Lys	Arg[b]	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

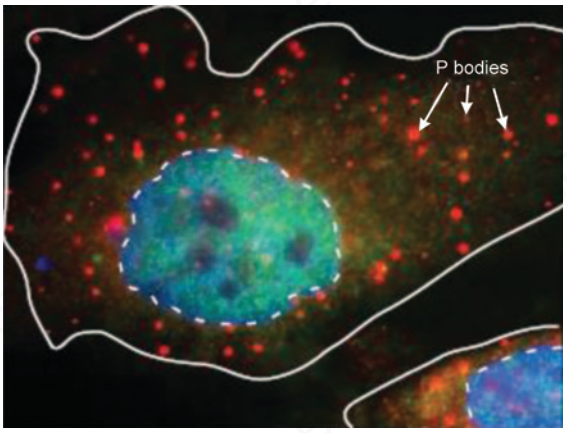
a. Frederick Sanger
b. Karry B Mullis
c. Robert Holley
d. Marshall Nirenberg

42. The components of the given initiation complex of translation include all except:



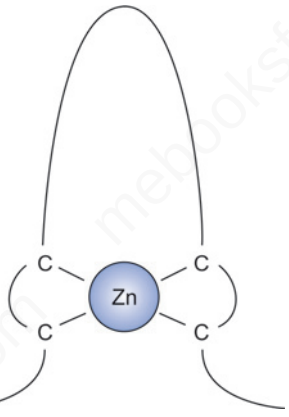
a. GTP
b. mRNA
c. 40S Subunit
d. 60S Subunit

43. What is the significance of the given organelle in the cell?



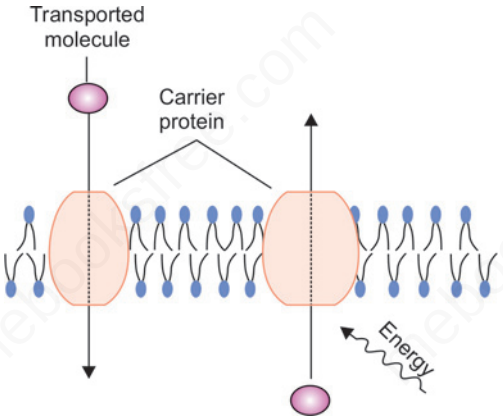
a. Posttranscriptional modification
b. mRNA degradation
c. Protein folding
d. Protein degradation

44. The given picture is an example of which organizational level of Protein?



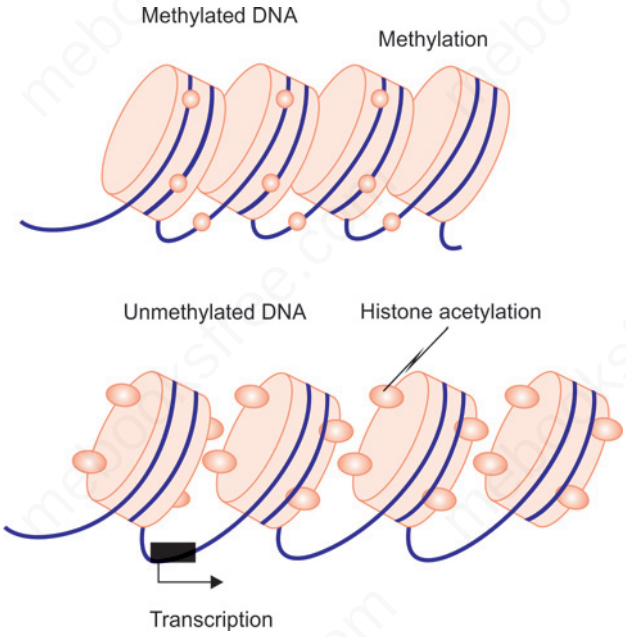
a. Primary structure
b. Secondary Structure
c. Super secondary structure
d. Tertiary structure

45. The transport mechanism depicted in the diagram is:



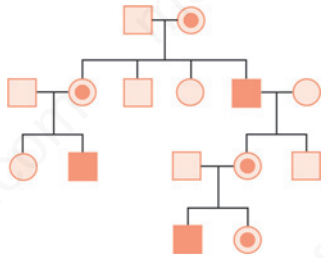
a. Simple diffusion
b. Facilitated diffusion
c. Ligand gated transport
d. Ion channels

46. What is the true statement about the regulatory mechanism of gene expression given in the picture?



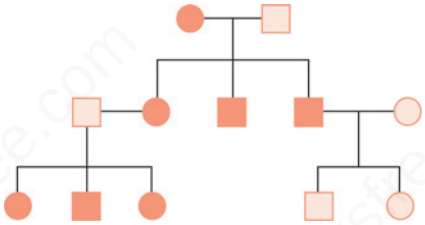
a. Posttranscriptional regulation
b. Affect mRNA stability
c. Alter the nucleotide sequence of the DNA
d. Alter organization of the DNA

47. **What is the inheritance pattern given in the diagram?**



a. Autosomal Dominant
b. Autosomal Recessive
c. X-linked Dominant
d. X-linked Recessive

48. **What is the inheritance pattern given in the diagram?**



a. Autosomal Dominant
b. Autosomal Recessive
c. Mitochondrial
d. X-linked Recessive

49. **Which of the following porphyria causes the given clinical findings?**



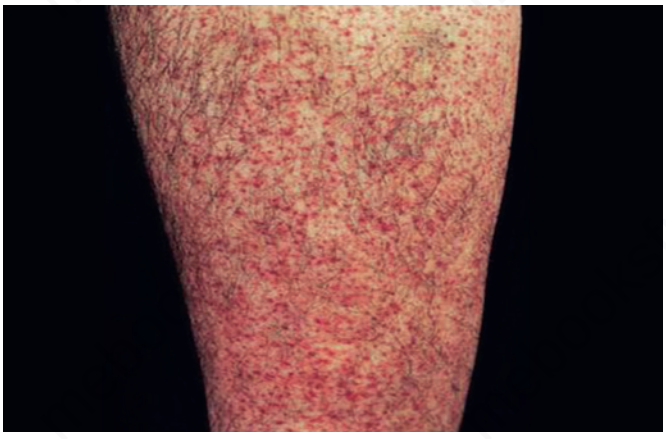
a. Acute Intermittent Porphyria
b. Variegate Porphyria
c. X-linked Protoporphyria
d. Porphyria Cutanea tarda

50. **A 5-year-old boy presented with swelling and redness in the dorsum of hands. A porphyria is diagnosed in this child. What is the enzyme deficiency that can lead to the given clinical picture?**



a. ALA Dehydratase
b. PBG Deaminase
c. Uroporphyrinogen Decarboxylase
d. Ferrochelatase

51. **Identify the vitamin deficiency given in the picture:**



a. Pellagra
b. Scurvy
c. Beriberi
d. Burning foot syndrome

52. The characteristic clinical finding in the picture is seen:



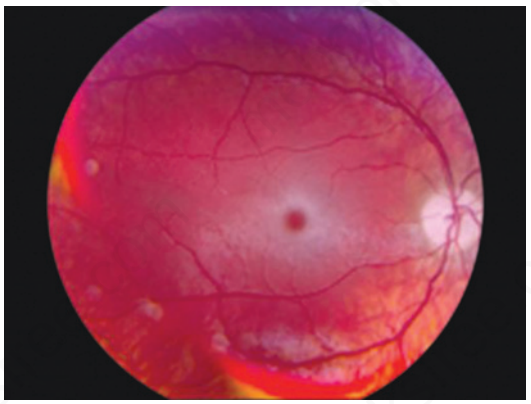
a. Familial Hypercholesterolemia
b. Familial Chylomicronemia
c. Abetalipoproteinemia
d. Broad Beta Disease

53. The characteristic clinical finding in the picture is seen:



a. Familial Hypercholesterolemia
b. Familial Chylomicronemia
c. Abetalipoproteinemia
d. Sitosterolemia

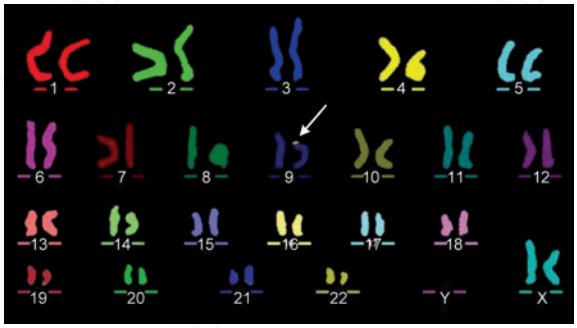
54. Which of the following metabolic disorder cannot present with the given clinical finding?



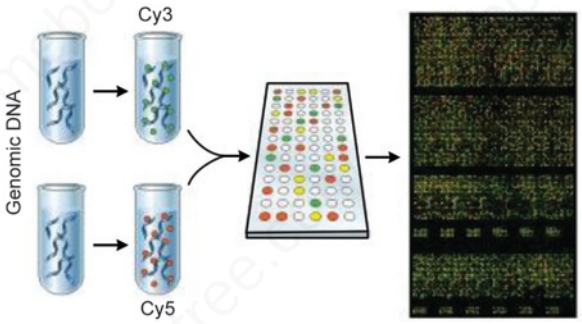
a. Tay-Sachs disease
b. Niemann Pick disease
c. Gaucher's disease
d. Sandhoff's disease

55. Which of the following regarding the cytogenetic technique is false:

a. Can diagnose Cri du chat disease
b. Can diagnose Philadelphia chromosome
c. Can be used to detect molecular defects in cancer
d. Unknown chromosomal anomaly can be detected

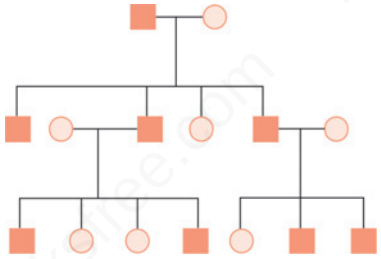


56. What is the chromosomal anomaly not detected by the technique given in the picture?



a. Copy number variations
b. Microdeletion
c. Translocations with no loss of genetic element
d. Amplification

57. What is the inheritance pattern?



a. Autosomal Dominant
b. X linked Dominant
c. Y Linked
d. Mitochondrial

58. What is the diagnosis of the given picture?



a. Pellagra
b. Beri beri
c. Rickets
d. Scurvy

59. Identify the clinical case kept for Final year MBBS OSCE examination in Pediatrics department:





a. Scurvy
b. Beriberi
c. Riboflavin deficiency
d. Pellagra

60. A 12-year-old male presented with multiple skeletal defects, dislocated lens, and a characteristic gait. What is the most probable enzyme defect?



a. Cystathionine Beta Synthase
b. Cystathionase
c. Methionine Synthase
d. Methylene THF Reductase

61. A 40-year-old male, presented to orthopedic OP with back pain. On examination the given clinical findings are noted. What is the enzyme deficiency that lead to the disorder?





a. Tyrosine Hydroxylase
b. Homogentisate Oxidase
c. Tyrosine Transaminase
d. Tyrosinase

62. This two-year-old girl presented with protuberant abdomen, coarse facial features, umbilical hernia, corneal clouding, mental retardation and short stature. What is the most probable diagnosis?



a. Hurler's disease
b. Hunter's disease
c. Moroteaux-Lamy disease
d. Scheie's disease

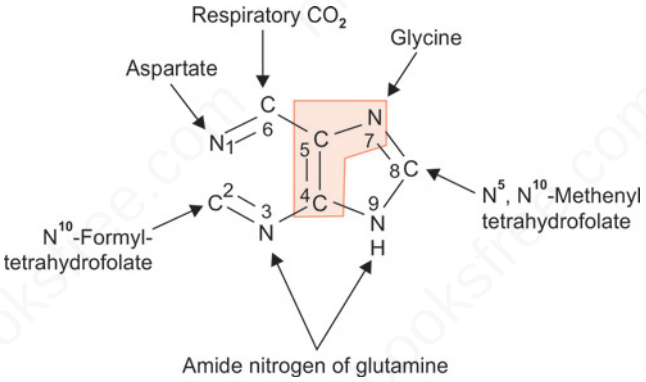
EXPLANATORY ANSWERS

1. **Ans. c.** Methionine
   - Sulphur containing amino acids are Cysteine and methionine
   - Thioether linkage is seen in Methionine
   - Thioalcohol group in Cysyteine
   - Cystine is a derived amino acid, formed by joining of two cysteine
   - Disulphide bond is present in Cystine.
2. **Ans. a.** Simple, Polar, Nonessential
   Glycine
   - Simple amino acid
   - Polar amino acid
   - Nonessential amino acid
   - Optically inactive amino acid.
3. **Ans. c.** Tyrosine
   - The amino acid given in the picture is Phenyl Alanine.
   - On hydroxylation form tyrosine.
   - The enzyme for this reaction is Phenylalanine Hydroxylase.
   - This enzyme belongs to class of Mono oxygenase.
   - The deficiency of this enzyme causes Phenyl Ketonuria.
4. **Ans. c.** Nonpolar imino acid
   - Proline
   - Imino acid
   - Nonpolar
   - Gives yellow colour to Ninhydrin test
   - Nonessential amino acid
5. **Ans. a.** Glutamate
   - Transamination reaction catalysed by Alanine amino Transferase (ALT)
   - Alanine is converted to Pyruvate.
   - Alpha Ketoglutarate is converted to Glutamate.
6. **Ans. d.** Vitamin B6
   - The given reaction is transamination.
   - PLP (Vitamin B6) is the coenzyme of the given reaction
7. **Ans. a.** Size Exclusion Chromatography
   - Size Exclusion chromatography separate the analytes based on the size.
   - Larger particles comes out first, smaller ones later.
8. **Ans. d.** Can also be used to study the secondary and tertiary structures of Proteins.
   Mass Spectrometry
   - Helps in sequencing of polypeptides, oligonucleotides and oligosaccharides.
   - Tandem Mass Spectrometry is the gold standard screening method for metabolic disorders.
   - Biological compounds are identified based on molecular mass or Mass to charge ratio
9. **Ans. c.** Cytochrome C Oxidase
   - Complex I (NADH CoQ Oxidoreductase) and Complex III (Q Cyt C Oxido Reductase) pumps 4 protons.
   - Complex II (Succinate CoQ Oxido Reductase) pumps no protons.
   - Complex IV (Cytochrome C Oxidase) pumps 2 protons
10. **Ans. b.** This structure can be translated to a protein
    - tRNA is a noncoding small RNA with 74 -95 nucleotide length.
    - Modified bases like Pseudouridine, Ribothymidine etc is present.
    - 3′ end has an acceptor arm with CCA base sequence.
    - Intrastrand base pairing is seen
11. **Ans. a.** DNA
    - Southern Blotting is the given technique. It can detect DNA.
12. **Ans. c.** This is an ***in vivo*** technique
    PCR
    - Is an *in vitro* amplification technique of nucleic acid invented by Dr Karry B Mullis
    - All the reaction carried out in single test tube.
    - Exponential amplification
    - Reverse transcriptase PCR can amplify RNA
13. **Ans. d.** β subunit of F1 subcomplex
    ATP Synthase
    - Otherwise called complex V of Electon transport chain
    - It has two subcomplexes Fo and F1
    - ATP is synthesized in the β subunit of F1.

- This is explained by binding change mechanism, put forward by Paul Boyer

14. **Ans. b.** Aconitase
    - Aconitase convert Citrate to Isocitrate, by a two step process.
    - Citrate to Cis Aconitate.
    - Cis aconitate to Isocitrate
    - This enzyme is inhibited by Fluoroacetate.
15. **Ans. c.** Tyrosine
    - Amino acid that enter at the level of Fumarate are Tyrosine and Phenyl Alanine
16. **Ans. d. 3** Phosphoglycerate
17. **Ans. b.** This is the major fate of glucose in RBC
    Rapaport Leubering Cycle
    - Takes place in the RBC
    - Substrate level phosphorylation at 1, 3 Bisphosphoglycerate Kinase is bypassed.
    - No net ATP when glucose enter this pathway.
    - This is not the major fate of Glucose in RBCs
    - The major fate of glucose in RBC is anaerobic glycolysis.
18. **Ans. b.** Muscle
    - The enzyme Glucose 6 Phosphatase is absent in the muscle.
19. **Ans. b.** Biotin
    Biotin is the coenzyme for carboxylation reaction
    - Propionyl CoA Carboxylase
    - Acetyl CoA Carboxylase
    - Pyruvate Carboxylase
20. **Ans. c.** Symptoms of Diabetes mellitus, but Blood glucose values are inconclusive:
    - The laboratory test is Oral Glucose tolerance test.
    - Indicated in doubtful cases of Diabetes Mellitus
21. **Ans. b.** Nonoxidative phase of HMP pathway
    Glucose 6 Phosphate Dehydrogenase
    - Rate limiting step of HMP Pathway
    - In irreversible oxidative phase of the pathway.
22. **Ans. b.** L-Gulanolactone Oxidase
    - Gulanolactone Oxidase is the enzyme in the uronic acid pathway, i.e. absent in humans and higher primates.
    - So Vitamin C is not synthesized in the above.
23. **Ans. d.** They are at increased risk of neonatal sepsis by *E. coli*.
24. **Ans. c.** Micelle
25. **Ans. b.** De Novo Fatty acid synthesis
    - Fatty acid Synthase Complex is a multi enzyme complex with 6 enzyme activities
    - Homo dimer wit X shape.
26. **Ans. a.** Monooxygenase
    - Phenylalanine Hydroxylase is a monooxygenase
27. **Ans. a.** Carnitine Palmitoyl Transferase I and Carnitine Palmitoyl Transferase II
    - CPT-1 in outer mitochondrial membrane and CPT-2 in Inner mitochondrial membrane
28. **Ans. d.** Acetone
    - Ketone body spontaneously formed from acetoacetate is Acetone.
29. **Ans. b.** This enzyme defect is the most common cause of Hyperammonemia
    - CPS-I is the enzyme
    - This the rate limiting step, takes place in the mitochondria.
    - This is an example of Biotin Independent carboxylation.
    - Most common cause of Hyperammonemia is Ornithine Transcarbamoylase defect.
30. **Ans. a.** Folic acid Deficiency
31. **Ans. d.** Strong oxidizing agent
    - Homogentisic acid is a strong reducing agent, hence give Benedict's test positive
32. **Ans. c.** Tryptophan
    - Kynurenine is an intermediate in Tryptophan metabolism
33. **Ans. d.** Vitamin B6
    Cytathionine Beta Synthase
    - Vitamin B6 is the coenzyme.
    - This enzyme deficiency causes Classic homocystinuria
34. **Ans. c.** Ornithine Decarboxylase
    - Spermine, Spermidine and Putrescine are Polyamines.
    - They are derived from Ornithine and Lysine.
    - Ornithine Decarboxylase is the rate limiting step
35. **Ans. a.** Biological rhythm
    - Functions of Melatonin are Biological rhythm and Diurnal Variation.
    - Synthesized in Pineal gland from Tryptophan.
36. **Ans. a.** Tyrosine
    - Compounds derived from Tyrosine are Melanin, Catecholamines (Epinephrine, Norepinephrine, Dopamine) and Thyroxine.

37. **Ans. b.** Aspartate
- Compounds that contribute to Purine ring



38. **Ans. d.** This takes place in the M phase of Cell cycle
- The picture depicts the Semiconservative model of DNA replication.
- Proved by Meselson and Stahl
- This takes place in the S phase of Cell cycle.
- This helps in passing the genetic information from parent to progeny.

39. **Ans. c.** DNA is wound in right handed direction
Nucleosome
- DNA Double helix wound on Histone octamer.
- This forms 10 nm Chromatin fibril, which has beads on string appearance.
- DNA is wound in left handed direction on histone octamer.
- 1.75 turns of DNA helix on histone octamer has around 145-150 bp

40. **Ans. b.** Promoters
RNA Polymerase Complex
- RNA Polymerase complex specifically binds to the promoters of transcription.
- σ factor helps in the binding of RNA Polymerase complex to prpmoters.

41. **Ans. d.** Marshall Nirenberg
Genetic Code
- Cracking of genetic code is done by Marshall Nirenberg and Har Gobind Khurana.

42. **Ans.** 60S Subunit
The figure given is 48S Initiation Complex
- GTP + eIF-2 + met tRNA = Ternary Complex
- Ternary Complex + 40S Ribosome = 43 S Pre initiation complex
- 43 S Pre initiation complex + mRNA = 48 S Initiation Complex

43. **Ans. b.** mRNA Degradation
P Bodies
- Nontranslating mRNA form ribonucleoprotein particles and they accumulate in cytoplasmic organelle called P bodies.
- Ribonucleoprotein or mRNP are mRNA, bound by specific packaging Proteins.
- P bodies are sites of translation repression and mRNA decay.
- P bodies contain mRNA decapping enzymes, RNA helicases, RNA exonucleases etc for mRNA quality control.
- A portion of miRNA driven mRNA modulation takes place in P bodies

44. **Ans. c.** Super secondary structures
- DNA binding motifs Zinc finger is given in the diagram
- It belongs to Supersecondary structures

45. **Ans. b.** Facilitated diffusion
- Facilitated diffusion is a carrier mediated passive transport

46. **Ans. d.** Alter organization of the DNA
The regulatory mechanism in the diagram is epigenetic modification.
- This does not alter the nucleotide sequence.
- The chemical modification of DNA or chromatin activate or inhibit gene expression.
- Activation of gene is by increasing euchromatin formation.
- Inactivation by heterochromatin formation.
- So it affects the organization of the DNA.

47. **Ans. d.** X-linked recessive
The characteristics of X-linked recessive inheritance.
- Males are usually affected.
- Females are usually carriers.
- Affected males will have only carrier females
- Carrier female will have affected males
- Male to male transmission is never seen

48. **Ans. c.** Mitochondrial
The Characteristics of Mitochondrial inheritance
- Females transmit the disease to all her offsprings.
- Males usually never transmit the disease.
- This is called Matrilinearinheritance.
- Other name is Cytoplasmic inheritance.

49. **Ans. d.** Porphyria Cutanea tarda

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Porphria Cutanea Tarda

Uroporphyrinogen Decarboxylase defect

80% is sporadic attributed to Uroporphyrinogen Decarboxylase inhibitors.

The aggravating factors of PCT are:

1. Hepatitis C, HIV
2. Excess Alcohol
3. Elevated Iron
4. Estrogen

- Most Common Porphyria
- Most readily treated Porphyria
- Associated with Hemochromatosis
- Blistering Skin Lesions mostly in the back of hands.
- They are susceptible to develop chronic liver disease and are at risk for Hepatocellular Carcinoma.

Treatment

- Repeated Phlebotomy to reduce hepatic iron.
- Low dose regimen of Chloroquine or Hydroxy chloroquine.
- In patients with end stage renal Disease, administer Erythropoietin.

50. Ans. d. Ferrochelatase

Erythropoietic Protoporphyria

Due to defect in Ferochelatase (FECH Mutation)

- Most common porphyria in children and second most common in adults.
- NON BLISTERING Photosensitivity
- Characterised by pain, swelling redness within minutes of sunlight exposure, resembling angioedema.
- Vesicular lesions are uncommon.

51. Ans. b. Scurvy

Scurvy

- Petechiae, ecchymosis, coiled hairs, inflamed and bleeding gums, joint effusion, poor wound healing, fatigue.
- Perifollicular hemorrhages
- Perifollicular hyperkeratotic papules, petechiae, purpura,
- Splinter hemorrhage, bleeding gums, hemarthroses, subperiosteal hemorrhage
- Anaemia
- Late stage are characterised by edema, oliguria, neuropathy, intracerebral hemorrhage and death

52. Ans. a. Familial hypercholesterolemia

The given picture is tendon xanthoma, usually seen in Type IIa Hyperlipoproteinemia

Clinical presentation of Familial Hypercholesterolemia

- Family history of premature CHD
- Corneal arcus
- Plasma is clear
- No pancreatitis
- Tendon xanthomas particularly dorsum of hands and Achilles tendon.
- Increased risk of cardiovascular disease.

53. Ans. b. Familial Chylomicronemia

The given picture is eruptive xanthoma usually seen in Type I and Type V Hyperlipoproteineimia

Clinical presentation of Familial Chylomicronemia Syndrome

- Present in childhood with recurrent abdominal pain due to acute pancreatitis
- On fundoscopic examination opalescent retinal blood vessels (lipemia retinalis)
- Lactescent plasma
- Eruptive xanthoma (small yellowish white papules appear in clusters on backs, buttocks, extensor surfaces of arms and legs. These are painless skin lesions may become pruritic)
- Hepatosplenomegaly
- Premature CHD is not a featureof FCS.

54. Ans. c. Gaucher's Disease

- This is the picture of Cherry red spot in the macula
- Sphingolipidosis with no Cherry red spot on macula-Gaucher's Type I, Fabry's Disease

55. Ans. d. Unknown chromosomal anomaly can be detected

Multicolor FISH

Uses of FISH

- Detection of numeric abnormalities of chromosomes (aneuploidy)
- The demonstration of subtle micro deletions
- Detection of complex translocations not detectable by routine karyotyping
- For analysis of gene amplification
- For mapping newy isolated genes[Q] of interest to their chromosomal loci.

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Disadvantages of FISH

- FISH requires a preselection of an informative molecular probe prior to analysis.
- So a prior knowledge of the anomaly is needed.

56. Ans. c. Translocations with no loss of genetic element

Array CGH

- Detect Gene Amplification
- Detect Gene Deletion.
- Detect Copy number variations

But Array CGH will not detect a balanced translocation, without loss of genetic elements

57. Ans. c. Y-linked

The characteristics of Y-linked inheritance

- Only males are affected
- Only male to male transmission is seen
- The explanation is simple only Y chromosome carries the mutant allele.

58. Ans. c. Rickets

- Rachitic rosary of Rickets is given in the picture.

59. Ans. c. Riboflavin deficiency

Deficiency manifestation of Vitamin B2 (Riboflavin)

Magenta tongue (Glossitis), angular stomatitis, **Seborrheic Dermatitis**, Cheilosis, **Corneal vascularization**, anemia

60. Ans. a. Cystathionine Beta Synthase

Clinical Features of Classic Homocystinuria

- Normal at birth
- Symtoms during infancy are nonspecific and may include failure to thrive and developmental delay.
- The diagnosis is usually made after 3 years of age, when subluxation of the ocular lens (ectopia lentis) occurs. This causes severe myopia and iridodonesis (quivering of the iris).
- Progressive **intellectual disability** is common
- **Skeletal abnormalities** resembling those of **Marfan syndrome** tall and thin, with elongated limbs and arachnodactyly scoliosis, pectus excavatum or carinatum, genu valgum, pes cavus, high-arched palate, and crowding of the teeth are commonly seen.
- These children usually have fair complexions, blue eyes, and a peculiar malar flush
- **Thromboembolic episodes** involving both large and small vessels, especially those of the brain, are common and may occur at any age

61. Ans. b. Homogentisate Oxidase

Alkaptonuria

Clinical Presentation of Alkaptonuria

- Normal Life till 3rd or 4th decade.
- Urine Darkens on standing is the only manifestation in children.
- In adults **Ochronosis** i.e. Alkaptone Bodies deposited in Intervertebral Disc, cartilage of nose, pinna etc leading to pigmentation.
- Arthritis
- NO MENTAL RETARDATION[Q]

62. Ans. a. Hurler's disease

- Option B-The case given here is a girl and there is corneal clouding so it is not Hunter's disease.
- Option C and D –There is no mental retardation in Moroteaux-Lamy Disease, Scheie's disease.

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