EDO UNIVERSITY IYAMHO
Department of Biochemistry

BCH 314: Amino acid, Protein and Nucleic acid Metabolism

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General overview of the Course

The course covers the following topic: Detailed treatment of metabolism of amino acids, degradation and biosynthesis. Oxidative and non-oxidative deamination, transamination and decarboxylation, transamidation. Disorders of amino acid metabolism. Inborn errors of metabolism. Metabolism of inorganic nitrogen. Transport and toxicity of ammonia. The urea cycle. Creatine metabolism. Polyamines. Nucleoside, nucleotide and nucleic acid synthesis and degradation. One carbon metabolism. Transmethylation. Disorders of nucleotide metabolism. Hyperuricemia and other inborn errors. Protoporphyrin synthesis in animals and plants. Hormone and regulatory role inintermediary metabolism.

Intended Learning Outcomes

At the end of this aspect of the course, students should be able to

1. Discuss the metabolism of amino acids
2. Discuss the different processes involved in the metabolism of amino acids
3. Relate derangements in the metabolism of amino acids as well as inborn errors of metabolism.
4. Discuss the transport and toxicity of ammonia.
5. Discuss the urea cycle
6. Discuss creatine biosynthesis

7. Discuss the metabolism of nucleoside, nucleotide and nucleic acids
8. The disorders of nucleotide metabolism
9. Explain one carbon metabolism

Assignments: We expect to have 5 individual homework assignments throughout the course in addition to a Mid-Term Test and a Final Exam. Home works are due at the beginning of the class on the due date. Home works are organized and structured as preparation for the midterm and final exam, and are meant to be a studying material for both exams. There will also be 2 term papers are expected to be written by individuals taking this course. This is aimed at broadening students; knowledge of the course.

Grading: We will assign 10% of this class grade to home works, 10% for the term papers, 10% for the mid-term test and 70% for the final exam. The Final exam is comprehensive.

Textbooks: The recommended textbooks for this class are as stated:

Title: *Lehninger Principles of Biochemistry*
Authors: David L. Nelson, Michael M. COX
Publisher: Freeman, W. H. and Company, Seventh Edition
ISBN- 13: 9787464126116
Year: 2017

Title: *Textbook of Medical Biochemistry*
Author(s): MN Chatterjea and RanaShinde.
Publisher: Jaypee Brothers Medical Publishers Ltd, Eighth Edition
ISBN: 978-93-5025-484-4
Year: 2012

Title: *Textbook of biochemistry for Medical Students*
Author(s): DM Vasudevan, Sreekumari S and KannanVaidyanathan.
Publisher: Jaypee Brothers Medical Publishers Ltd, Sixth Edition
ISBN: 978-93-5025-016-7
Year: 2011

Title: Lippincottôs Illustrated Reviews Biochemistry
Author: Denise R. Ferrier
Publisher: Lipincott Williams & Wilkins
ISBN: 978-1-4511-7562-2
Year: 2014

Title: Harperôs Illustrated Biochemistry. 28th edition
Authors: Robert K. Murray, Daryl K. Granner, Victor W. Rodwell
Publisher: McGraw Hill Lange

Title: Essential Cell Biology
Authors: Alberts Bray, Hopkin Johnson, Lewis Raff, Roberts Walter
Publisher: Garland Science
Year: 2004

MAIN LECTUTRE

Amino acid digestion
Most of the nitrogen in the diet is consumed in the form of protein, typically amounting to 70ï 100 g/day in the diet. Proteins are generally too large to be absorbed by the intestine. An example of an exception to this rule is that newborns can take up maternal antibodies in breast milk. They must, therefore, be hydrolyzed to yield di- and tripeptides as well as individual amino acids, which can be absorbed. Proteolytic enzymes responsible for degrading proteins are produced by three different organs: the stomach, the pancreas, and the small intestine. In the stomach, pepsin is the major proteolytic enzyme. It cleaves proteins to smaller polypeptides. Pepsin is produced and secreted by the chief cells of the stomach as the inactive zymogen pepsinogen.

Hydrochloric acid (HCl) produced by the parietal cells of the stomach causes a conformational change in pepsinogen that enables it to cleave itself (autocatalysis), forming active pepsin. Pepsin has a broad specificity but tends to cleave peptide bonds in which the carboxyl group is contributed by the acidic amino acids, aromatic amino acids, or leucine. In the intestine, the partially digested material from the stomach encounters pancreatic secretions, which include bicarbonate and a group of proteolytic enzymes. Bicarbonate neutralizes the stomach acid, raising the pH of the contents of the intestinal lumen into the optimal range for the digestive enzymes to act.

Endopeptidases from the pancreas cleave peptide bonds within protein chains. Trypsin cleaves peptide bonds in which the carboxyl group is contributed by arginine or lysine. Trypsin is secreted as the inactive zymogen trypsinogen. Trypsinogen is cleaved to form trypsin by the enzyme enteropeptidase (enterokinase), which is produced by intestinal cells. Trypsinogen may also undergo autocatalysis by trypsin. Chymotrypsin usually cleaves peptide bonds at the carboxyl group of aromatic amino acids or leucine. Chymotrypsinogen, the inactive zymogen, is cleaved to form active chymotrypsin by trypsin.
Elastase cleaves at the carboxyl end of amino acid residues with small, uncharged side chains such as alanine, glycine, or serine. Proelastase, the inactive zymogen, is cleaved to active elastase by trypsin. Exopeptidases in the pancreas (carboxypeptidases A and B) cleave one amino acid progressively from the C-terminal end of the peptide.

The carboxypeptidases are produced as inactive procarboxypeptidases, which are cleaved to their active form by trypsin. Carboxypeptidase A cleaves aromatic amino acids from the C terminus.
Carboxypeptidase B cleaves the basic amino acids, lysine and arginine, from the C terminus.

Proteases produced by intestinal epithelial cells complete the conversion of dietary proteins to peptides and finally to amino acids. Aminopeptidases are exopeptidases produced by intestinal cells, cleaving one amino acid at a time from the N terminus of peptides. Dipeptidases and tripeptidases associated with the intestinal cells produce amino acids from dipeptides and tripeptides.

Amino acid absorption

Amino acids resulting from protein digestion are absorbed from the small intestine by:

1. Passive transport mechanism (For D-amino acids).
2. Active transport mechanism (For L-amino acids and dipeptides), and this occurs via;
3. Carrier protein transport system

(sodium ï amino acid carrier system).

4. Glutathione transport system

(ɔ-glutamyl cycle)

Carrier protein transport system (sodium ï amino acid carrier system)

This system transport the amino acids against its concentration gradient using energy derived from Na/K+ pump. Here, amino acids are absorbed by specific carrier protein in the cell membrane of the small intestinal cells. This carrier protein has one site for the amino acids and another site for the Na+.

It transports them from the intestinal lumen across the cell membrane to the cytoplasm.

Then, the amino acid passes to the blood down its concentration gradient, while the Na+ is pumped out from the cell to the intestinal lumen by Na/K+ pump utilizing ATP as a source of energy derived from Na/K+ pump.

Glutathione transport system (ɔ-glutamyl cycle)

This transport system is for the transport of amino acids from the extracellular space to the cytoplasm in the intestine, kidney, brain & liver (bile ductile cells).

An amino acid in the lumen reacts with glutathione (g-glutamyl-cysteinyl-glycine) in the cell membrane, forming a g-glutamyl amino acid and the dipeptide cysteinyl-glycine.

The amino acid is carried across the cell membrane attached to g-glutamate and released into the cytoplasm. The g-glutamyl moiety is used in the resynthesis of glutathione.

In infants, Ig A in the clostrum of milk is absorbed without digestion by pinocytosis, thereby giving immunity to the babies.

Amino acid degradation

There are 3 common stages of amino acid degradation:

Deamination; the removal of amino group(s) which are converted into:

ammonia or the amino group of aspartate.

Incorporation of ammonia or aspartate nitrogen into urea for excretion.

Conversion of the amino acid carbon skeletons (i.e. the a-keto acids that result from deamination) to the common intermediates.

The first step in amino acid breakdown usually is removal of an alpha-amino group and it is achieved through the following processes;

1. Transamination
2. Deamination
3. Oxidative deamination

4. Non-oxidative deamination
5. Transdeamination

Specific methods of deamination, which applies to some specific amino acids.

Transamination

It is the transfer of amino group from one Ŭ- a.a. to Ŭ- keto acid to form a new Ŭ- amino acid & a new Ŭ- keto acid.

Transamination reactions are readily reversible and can be used in the synthesis or the degradation of amino acids.

The process represents only an intermolecular transfer of NH2 group without the splitting out of NH3. Ammonia formation does not take place by transamination reaction.

Enzymes involved:

Transaminases or aminotransferases

Coenzyme: PLP (Pyridoxal phosphate)

Transamination occurs in two stages:

Transfer of the amino group to the coenzyme pyridoxal phosphate (bound to the coenzyme) to form pyridoxamine phosphate The amino group of pyridoxamine phosphate is then transferred to a keto acid to produce a new amino acid and the enzyme with PLP is regenerated. Transamination takes place in the cytosol or both the cytosol & the mitochondria of most cells especially in the liver, kidney, heart and brain.

But the enzyme is present in almost all mammalian tissues and transamination can be carried out in all tissues to some extent.

All amino acids except threonine, lysine, proline and hydroxyproline may undergo transamination. As an example, amino group is interchanged between alanine and glutamic acid. In almost all cases, the amino group is accepted by alpha ketoglutaric acid so that glutamic acid is formed. Ŭ-ketoglutarate& glutamate are often involved in transamination reactions.

Clinical importance of transamination

Function of transaminases:

Degradation of a.as to form Ŭ- keto acids.

Synthesis of non essential a.as from CHO.

Diagnostic value:

Transaminases are normally intracellular enzymes. They are elevated in the blood when damage to the cells producing these enzymes occurs.

Increased level of both ALT & AST indicates possible damage to the liver cells.

Increased level of AST alone suggest damage to heart muscle, skeletal muscle or kidney.

Deamination

The removal of an amino group from the amino acids as NH3 is deamination.

Deamination results in the liberation of ammonia for urea synthesis. Simultaneously, the carbon skeleton of amino acids is converted to keto acids.

Although transamination and deamination are separately discussed, they occur simultaneously, often involving glutamate as the central molecule. For this reason, some authors use the term transdeamination while describing the reactions of transamination and deamination, particularly involving glutamate.

Deamination is of two types;

1. Oxidative deamination and
2. Non-oxidative deamination

Oxidative deamination

Oxidative deamination is the oxidation(removal of hydrogen) and deamination (removal of the amino group which is liberated as free ammonia) giving Ŭ- ketoacid and ammonia (reversible reactions). Oxidative deamination takes place mostly in the liver and kidney. The purpose of oxidative deamination is to provide NH3 for urea synthesis and Ŭ-keto acids for a variety of reactions, including energy generation.

Regulation: The direction of the reaction depends on:

Availability of the substrates:

--Relative concentration of (Ŭ-ketoglutarate&NH3) and (glutamate).

--Ratio of NADP : NADPH+H

Allosteric regulation:

--Activators : ADP or GDP.

-- Inhibitors : ATP ,GTP & NADH

D- & L- Amino acid oxidases :

Present only in the liver and kidney in minimal amounts.

They are of low activity in the mammalian tissue

N.B: L-amino acids: mammalian proteins are formed of only L-amino acids. D-amino acids are found in plants and the cell wall of microorganisms but not used in the synthesis of mammalian proteins. L-amino acid oxidase deaminates most of the naturally occuring amino acids. D-amino acid oxidase deaminates D-amino acids present in diet giving Ŭ- keto acids that either transaminated to the coressponding L-amino acid or converted to glucose or fatty acids or catabolized to CO2 + H2O + energy.

Clinical importance of oxidative deamination

L-glutamate dehydrogenase enzyme is the only enzyme that undergoes oxidative deamination in the mammalian tissue.

Oxidative deamination by L- glutamate dehydrogenase is an essential component of transdeamination.

So, it is important in deamination of most amino acids.

L-Amino acid oxidase and D-amino acid oxidase are flavoproteins, possessing FMN and FAD respectively. They act on the corresponding amino acids (L or D) to produce a-keto acids and NH3. In this reaction, oxygen is reduced to H2O2, which is later decomposed by catalase.

Non-oxidative deamination

There are certain amino acids, which can be non-oxidativelydeaminated by specific enzymes, and can form NH3. These reactions do contribute to NH3 formation, but they do not fulfill a major role in NH3 formation.

Examples of non-oxidative deamination include:

Deamination of histidine: Histidine is non-oxidativelydeaminated by the specific enzyme Histidase to form NH3 and urocanic acid.

Amino acid dehydrases: The hydroxy amino acids viz serine, threonine and homoserine are deaminated by specific enzymes, called amino acid dehydrases which requires Pyridoxal-P (B6-P) as coenzyme. The enzymes catalyze a primary dehydration followed by spontaneous deamination.

Amino acid desulfhhydrases: S-containing amino acids, e.g. cysteine, and homocysteine are deaminated by a primary desulfhydration (removal as H2S), forming an imino acid, which is then spontaneously hydrolysed.

Transdeamination

It is the combination of transamination & oxidative deamination.

It includes the transamination of most amino acids with Ği ketoglutarate to form glutamate, then the glutamate is oxidativelydeaminated reforming Ği ketoglutarate and giving ammonia.

This provides a pathway by which the amino group of most amino acids is released in the form of ammonia.

The amino group of most of the amino acids is released by a coupled reaction, transdeamination, that is transamination followed by oxidative deamination.

Transamination takes place in the cytoplasm of all the cells of the body; the amino group is transported to liver as glutamic acid which is finally oxidativelydeaminated in the mitochondria of hepatocytes.

Thus, the two components of the reaction are physically far away, but physiologically they are coupled. Hence, the term trans-deamination.

Decarboxylation

Decarboxylation is the reaction by which CO2 is removed from the COOH group of an amino acid as a result an amine is formed.

The reaction is catalysed by the enzyme decarboxylase, which requires pyridoxal-P (B6-PO4) as coenzyme.

Tissues like liver, kidney, brain possess the enzyme decarboxylase and also by microorganisms of intestinal tract.

The enzyme removes CO2 from COOH group and converts the amino acid to corresponding amine. This is mostly a process confined to putrefaction in intestines and produces amines.

Examples of biogenic amines are Histamine (from histidine), GABA (from glutamic acid), ethanolamine (from serine), taurine (from cysteic acid), putrescine (from ornithine)

Polyamines

Polyamines are putrescine, spermidine and spermine.

They are aliphatic amines.

They are synthesized from Ornithine. Ornithine in addition to its role in urea cycle, serves as the precursor of ubiquitous mammalian and bacterial polyamines, spermidine and spermine. It requires ôactiveô methionine.

The key enzyme of polyamine synthesis is ornithine decarboxylase (ODC). It requires pyridoxal phosphate, and is induced by steroid hormones.

The enzyme,ODC has pyruvate (not PLP) as the prosthetic group; it is the only mammalian enzyme, known to contain bound pyruvate

Biochemical Functions of Polyamines

They have been implicated in diverse physiological processes and are involved in cell proliferation and growth. Putrescine is best ñmarkerò for cell proliferation.

They are required as ôgrowth factorsô for cultured mammalian and bacterial cells.

They have been implicated in the stabilisation of intact cells, sub-cellular organelles and membranes.
As Polyamines have multiple +ve charges, they can associate readily with Polyanions such as DNA and RNAs and have been implicated in such fundamental processes as stimulation of DNA and RNA biosynthesis, DNA stabilisation and packaging of DNA in bacteriophages.
Polyamines also exert diverse effects on protein synthesis.
They act as inhibitors of enzymes that include Protein kinases.
Polyamines added to cultured cells induce synthesis of a protein antienzyme that binds to ornithine decarboxylase and inhibits putrescine formation.
Spermidine has been claimed to be best ñmarkerò of tumor cell destruction.
In Pharmacologic dosage Polyamines have been found to be hypothermic and hypotensive.

Transport of ammonia
NH3 is absorbed from the intestine into portal venous blood which contains relatively high concentration of NH3 as compared to systemic blood.
Under normal conditions of health, Liver promptly removes the NH3 from the portal blood, so that blood leaving the liver is virtually NH3-free. This is essential since even small quantities of NH3 are toxic to CNS.
Two mechanisms are available in humans for the transport of ammonia from the peripheral tissues to the liver for its ultimate conversion to urea.
The first uses glutamine synthetase to combine ammonia with glutamate to form glutamine, a nontoxic transport form of ammonia. The glutamine is transported in the blood to the liver where it is cleaved by glutaminase to produce glutamate and free ammonia. The ammonia is converted to urea.
The second transport mechanism involves the formation of alanine by the transamination of pyruvate produced from both aerobic glycolysis and metabolism of the succinyl coenzyme A (CoA) generated by the catabolism of the branched-chain amino acids isoleucine and valine.
Alanine is transported by the blood to the liver, where it is converted to pyruvate, again by transamination.
The pyruvate is used to synthesize glucose, which can enter the blood and be used by muscle, a pathway called the glucoseï alanine cycle.
Thus, glutamic acid acts as the link between amino groups of amino acids and ammonia.
The concentration of glutamic acid in blood is 10 times more than other amino acids.
Glutamine is the transport forms of ammonia from brain and intestine to liver; while alanine is the transport form from muscle.
Glutamine removes the toxic effect of NH3 in the brain. Then the glutamine goes via the blood to the kidneys where it become hydrolyzed by glutaminase into glutamic acid and NH3 which is excreted in urine (This accounts for 60% of the NH3 excreted in urine)
NH3 produced from a.a. deamination in the kidney is directly excreted in urine (This accounts for 40% of NH3 excreted in urine)
N.B. NH3 produced from amino acid deamination in the kidney especially glutamine regulates acid base balance and preserve cations.

Toxicity of ammonia
An increase in blood NH3 concentration has adverse effect on the brain, as it produces the symptoms such as

Slurred speech, Blurred vision, Flapping tremor. Under severe states, coma and even death.
The reaction catalyzed by glutamate dehydrogenase probably explains the toxic affect of NH3 in brain. Accumulation of NH3 shifts the equilibrium to the right with more glutamate formation, hence more utilization of Ŭ-ketoglutarate. Ŭ- Ketoglutarate is a key intermediate in TCA cycle and its depleted levels impair the TCA cycle. The net result is that production of energy (ATP) by the brain is reduced. The toxic effects of NH3 on brain are therefore, due to impairment in ATP formation. Another reason is the fact that increased NH3 concentration enhances glutamine formation from Glutamate and thus reduces ôbraincellô pool of glutamic acid. Hence there is decreased formation of inhibitory neurotransmitter GABA (ɔ-aminobutyric acid).

Urea cycle

The ammonium ion, the end product of amino acid degradation, is toxic if it is allowed to accumulate. The urea cycle converts ammonium ions to urea, which is transported to the kidneys to form urine. The urea cycle in the liver cells consists of reactions that occur in the mitochondria and cytosol. detoxifies ammonium ions from amino acid degradation. begin with the conversion of ammonium ions to carbamoyl phosphate using energy from two ATP. The enzymes that catalyze the reactions are located partly in the mitochondria and partly in the cytosol.
The reactions of urea cycle can be studied in five sequential enzymatic reactions.
Reaction 1: Synthesis of carbamoyl-phosphate
Reaction 2: Synthesis of citrulline
Reaction 3: Synthesis of argininosuccinate
Reaction 4: Cleavage of argininosuccinate
Reaction 5: Cleavage of arginine to form ornithine and urea
The urea cycle is a cyclic process and the five reactions involve ornithine, citrulline, arginine and aspartic acid.
Urea formation takes place in liver in mammals and all of the enzymes involved have been isolated from Liver tissue.

Reaction 1: Synthesis of Carbamoyl-P (Mitochondrial)

In this reaction, HCO_3^- , NH_4^+ and phosphate derived from ATP reacts to form carbamoyl-P (also called Carbamyl-P). The reaction is catalysed by the mitochondrial-enzyme Carbamoyl phosphate synthetase 1. Mitochondrial carbamoyl phosphate synthetase I catalyses the ATP-dependant conversion of HCO_3^- and NH_4^+ to the energy-rich, mixed anhydride carbamoyl phosphate.

Reaction 2: Synthesis of Citrulline: (Mitochondrial)

In reaction 2, the enzyme, Ornithine transcarbamoylase which is also known as ornithine carbamoyltransferase is found associated with carbamoylphosphatesynthetase I in the mitochondrial matrix. It catalyses the nucleophilic addition of ornithine to the carbonyl group of carbamoyl-P to produce Citrulline. During this reaction, the ŭ-NH_2 group of ornithine attaches to the carbonyl group of carbamoyl-P and the phosphate group (Pi) is released. It is important to note that Ornithine which is used up at this stage of the reaction, is regenerated in cytosol in the 5th reaction and transported into the mitochondrial matrix by a specific transport protein in the inner mitochondrial membrane.

Reaction 3: Synthesis of Argininosuccinate: (cytosolic):

In reaction 3, the Citrulline which is produced in mitochondrial matrix in reaction 2, is transported across the inner mitochondrial membrane to the cytosol by a specific transport

protein. It then condenses with Aspartate to form argininosuccinate in an ATP-dependant reaction catalysed by argininosuccinatesynthetase. During this reaction, transfer of an ñadenylylò group from ATP to citrulline generates the activated intermediate ñCitrullyl-AMPò. Formation of the citrullyl- AMP intermediate facilitates removal of the ureido oxygen (carbonyl oxygen) of citrulline. The isoureido carbon of citrullyl-AMP is subjected to nucleophilic attack by the Ŭ-NH2 group of aspartate. The isoureido oxygen leaves with the departing AMP, and ócargininosuccinateô is formed

Reaction 4: Cleavage of Argininosuccinate: (Cytosolic)

In this reaction of urea cycle, the enzyme argininosuccinase also known as ArgininosuccinateLyasecatalyses conversion of Argininosuccinate to arginine and fumarate.

The urea cycle is linked to the TCA cycle through the production of fumarate. Amino acid catabolism, is therefore directly coupled to energy production. The fumarate is converted to oxaloacetate (OAA) via the fumarase and malate dehydrogenase reactions and then transaminated to regenerate aspartate to participate in the cycle.

Reaction 5: Cleavage of Arginine to Ornithine and Urea

The last reaction of the urea cycle completes the cycle. It is catalysed by the enzyme arginase, which is found only in the liver cells. Arginase catalyses hydrolysis of the guanidine group of arginine, releasing urea and regenerating ornithine. Ornithine now enters mitochondrion through inner mitochondrial membrane by a specific transport protein. Ornithine and lysine are potent inhibitors competitive with arginine.

Highly purified arginase from mammalian liver cells is activated by CO++ and Mn++.

Bioenergetics of the urea cycle

Urea cycle consumes four "high-energy" phosphate bonds (3 ATP hydrolyzed to 2 ADP and one AMP).

1 ATP	ADP + Pi
1 ATP	ADP + Pi
1 ATP	AMP + Pi + Pi

However, One NADH+H molecule is produced by oxidative deamination of glutamate to NH3 and Ŭ-ketoglutarate. Glutamate provides the NH3 used in the initial synthesis of carbamoyl phosphate. Also fumarate in the cycle may be converted to malate in the cytosol . Malate then oxidized to oxaloacetate gives 1 NADH+H equivalent to 3 ATP obtained from 3ADP,

So the net energy expenditure is only one high energy phosphate. The two NADH+H produced can provide energy for the formation of 5 ATP, a net production of one high energy phosphate bond for the urea cycle. However, if gluconeogenesis is underway in the cytosol, the latter reducing equivalent is used to drive the reversal of the glyceraldehyde 3-p dehydrogenase step instead of generating ATP. So the net energy expenditure is only one high energy phosphate .

Regulation of the urea cycle

The first reaction catalysed by carbamoyl phosphate synthase l is the rate limiting reaction or committed step in urea synthesis.

Carbamoyl phosphate synthase I is allosterically activated by N-acetylglutamate which is synthesized from glutamate and acetyl CoA by synthase and degraded by a hydrolase.

The rate of urea synthesis in liver is correlated with the concentration of N-acetylglutamate and this could be directly affected by:

Intake of protein-rich diet. Concentration of arginine

Excretion of urea

After the formation of urea in the liver, it diffuses into blood and is transported to the kidneys for excretion. However, a small proportion of the urea produced, enters the intestine and is degraded to carbon dioxide and ammonia by an intestinal bacterial enzyme called urease. The resultant ammonia is either lost in the faeces or absorbed into the blood. Patients with renal failure have problems excreting urea, hence there is a buildup of urea in the blood. A condition known as uremia. In uremia, there is diffusion of more urea into the intestine with resultant breakdown to ammonia. This results in elevated blood ammonia (hyperammonemia)

Consequently, renal failure patients are administered oral antibiotics to kill the intestinal bacteria.

Clinical significance of urea

The normal blood urea concentration in a healthy adult is 10-40mg/dl. However, this value could increase within normal range with high protein intake.

About 15-30 g of urea (7-15 g nitrogen) is excreted in urine per day.

Blood urea estimation is widely used as a screening test for the evaluation of renal function.

Elevation in blood urea may be broadly classified into three categories:

Prerenal causes:

These are conditions associated with increased protein breakdown or reduced plasma volume/body fluids with resultant negative nitrogen balance. Examples include

Salt and water depletion, diabetic coma, severe and prolonged diarrhoea, thyrotoxicosis, pyloric stenosis with severe vomiting, Haematemesis, Haemorrhage and shock; shock due to severe burns, Ulcerative colitis with severe chloride loss. Severe and protracted vomiting as in pyloric and intestinal obstruction,

Renal causes

The blood urea can be increased in all forms of kidney diseases:

In acute glomerulonephritis.

In early stages of type II nephritis (nephrosis) the blood urea may not be increased, but in later stages with renal failure, blood urea rises.

Other conditions are

malignantnephrosclerosis, chronic pyelonephritis and mercurial poisoning.

In diseases such as hydronephrosis, renal tuberculosis; small increases are seen but depends on extent of kidney damage.

Postrenal causes

These lead to increase in blood urea, when there is obstruction to urine flow. This causes retention of urine and so reduces the effective filtration pressure at the glomeruli; when prolonged, produces irreversible kidney damage. Causes include:

Enlargement of prostate, Stones in urinary tract, Stricture of the urethra, Tumours of the bladder affecting urinary flow.

Inborn errors of amino acid metabolism

Inborn errors of metabolism result from the synthesis of abnormal proteins, specifically enzymes which are often caused by mutant genes.

If an error occurs in the gene that codes for the enzyme a FAULT occurs.

Subsequently, the enzyme is not produced and the pathway breaks down.

Inborn errors of metabolism are uncommon but complicated medical conditions involving abnormalities in complex biochemical and metabolic pathways
The inherited defects may be expressed as a total loss of enzyme activity or, more frequently, as a partial deficiency in catalytic activity. Examples of such are:
Phenylketonuria
Tyrosinosis or Tyrosinemia type I
Tyrosinemia type II
Alkaptonuria
Albinism
Maple syrup urine disease

Phenylketonuria
Phenylketonuria(PKU) is the most common metabolic disorder in amino acid metabolism.
The name phenylketonuria was derived from the metabolite, phenylpyruvate which is a keto acid (C6H5CH2-CO-COO-) excreted in urine in high amounts in PKU. lt is due to the deficiency of the hepatic enzyme, phenylalanine hydroxylase, caused by an autosomal recessive gene. This enzyme deficiency impairs the synthesis of tetrahydrobiopterin required for the action of phenylalanine hydroxylase. The net outcome in PKU is that phenylalanine is not converted to tyrosine. Phenylketonuria primarily causes the accumulation of phenylalanine in tissues and blood, and results in its increased excretion in urine. Due to disturbances in the routine metabolism, phenylalanine is diverted to alternate pathways, resulting in the excessive production of phenylpyruvate, phenylacetate, phenyllactate and phenylglutamine. All these metabolites are excreted in urine in high concentration in PKU. Phenylacetate gives the urine a mousey odour.

Clinical features
Elevated phenylalanine:
Phenylalanine is present in high concentrations (ten times normal) in tissues, plasma, and urine. Phenyllactate, phenylacetate, and phenylpyruvate
Central nervous system symptoms:
Severe intellectual disability, developmental delay, microcephaly, and seizures are characteristic findings in untreated PKU.
Hypopigmentation:
Patients with untreated PKU may show a deficiency of pigmentation (fair hair, light skin color, and blue eyes).

Diagnosis
By estimation of plasma Phenyl alanine level.
By screening for the presence of phenyl pyruvate with FeCl3 (In urine).
Administration of phenyl alanine to a phenylketonuric patient should result in prolonged elevation of the level of this amino acid in blood (ñphenyl alanine tolerance testò).

Treatment
Most natural protein contains phenylalanine, an essential amino acid, and it is impossible to satisfy the bodyôs protein requirement without exceeding the phenylalanine limit when ingesting a normal diet. Therefore, in PKU, blood phenylalanine level is maintained close to the normal range by feeding synthetic amino acid preparations free of phenylalanine, supplemented with some natural foods.

Tyrosinosis or Tyrosinemia type I

Tyrosinosis is a rare inherited disorder that is characterised by accumulation of metabolites that adversely affect the activities of several enzymes and transport systems. In tyrosinosis, there is lack of the enzyme Fumaryl acetoacetate hydrolase and possibly also Maleyl acetoacetate isomerase.

Tyrosinosis could be acute or chronic.

In acute tyrosinosis, infants exhibit diarrhoea, vomiting, a ñcabbageò-like odour. They do not thrive well, and there is usually associated Liver damage. Infants die from liver failure. Untreated acute tyrosinosis cases do not survive and death occurs within 6 to 8 months.

In chronic tyrosinosis:

Clinical features are similar but milder symptoms and course. Children survive and in untreated cases leads to death by the age of 10 years. In both types plasma tyrosine levels are elevated: 6 to 12 mg/dl. There also occurs increase in plasma methionine level.

Treatment:

Involves a diet low in phenyl alanine and tyrosine and sometimes also low in methionine.

Tyrosinemia II

This disorder is also known as Richner-Hanhart syndrome and it is due to a defect in the enzyme tyrosine transaminase.

This results in a blockade in the routine degradative pathway of tyrosine. This results in the accumulation and excretion of tyrosine and its metabolites, namely p-hydroxyphenylpyruvate, p-hydroxyphenyllactate, phydroxyphenylacetate, N-acetyltyrosine-and tyramine are observed.

Clinical findings include:

Mental retardation, which may be mild to moderate.

Skin lesions (dermatitis) and eye lesions.

Some infants may exhibit self-mutilation and disturbances in fine co-ordination

There is an elevation of plasma tyrosine level

Tyrosine as well as the metabolites, tyramine and N-acetyltyrosine are excreted in urine

Treatment:

Involves a diet low in phenyl alanine and tyrosine

Neonatal Tyrosinemia

Neonatal tyrosinemia is caused by the deficiency of the enzyme p-hydroxyphenylpyruvatedioxygenase.

It may be seen in premature infants.

Blood levels of tyrosine and phenyl alanine are elevated.

Urinary excretion of tyrosine, tyramine, p-OH-Phenyl acetate, and N-acetyl tyrosine are increased.

Treatment:

Involves feeding a diet low in protein, specially with low phenyl alanine and tyrosine.

Neonatal tyrosinemia is mostly a temporary condition and usually responds to ascorbic acid especially in premature infants.

It is explained that the substrate inhibition of the enzyme is overcome by the presence of ascorbic acid.

Alkaptonuria

Alkaptonuria is also known as the black urine disease. A rare inborn error or hereditary defect in metabolism of Phenyl alanine and Tyrosine. It is due to the deficiency of homogentisate oxidase. Homogentisate accumulates in tissues and blood, and is excreted into urine. Homogentisic acid like many derivatives of tyrosine is readily oxidised to black pigments (alkapton). Hence, the urine of alkaptonuric patients when exposed to air slowly turns black from top to bottom. Alkapton deposition occurs in connective tissue, bones and various organs (nose, ear etc.) resulting in a condition known as ochronosis. Many alkaptonuric patients suffer from arthritis and this is believed to be due to the deposition of pigment alkapton (in the joints), produced from homogentisate.

Treatment:

Alkaptonuria is not a life-threatening condition. Hence, no specific treatment is required. However, consumption of protein diet with relatively low phenylalanine content is recommended.

Albinism

Albinism refers to a group of conditions in which a defect in tyrosine metabolism results in a deficiency in the production of melanin.

The most common cause of albinism is a defect in tyrosinase, the enzyme most responsible for the synthesis of melanin.

These defects result in the partial or full absence of pigment from the skin, hair, and eyes.

There are various forms of the disease. But can be divided into two major groups:

Oculocutaneous albinism

Ocular albinism

Clinical features:

The most important function of melanin is the protection of the body from sun radiation. Lack of melanin in albinos makes them sensitive to sunlight.

Increased susceptibility to skin cancer (carcinoma) is observed.

Photophobia (intolerance to light) is associated with lack of pigment in the eyes. However, there is no impairment in the eyesight of albinos.

Maple syrup urine disease

Maple syrup urine disease (MSUD) is a rare autosomal recessive disorder in which there is a partial or complete deficiency in branched-chain Ŭ-keto acid dehydrogenase (BCKD), a mitochondrial enzyme complex that oxidativelydecarboxylatesleucine, isoleucine, and valine.

The name originates from the characteristic smell of urine (similar to burnt sugar or maple sugar) due to excretion of branched chain keto acids.

Clinical features:

Disease starts in the first week of life.

It is characterized by convulsions, severe mental retardation, vomiting, acidosis, coma and death within the first year of life.

Treatment:

Treatment is achieved by the ingestion of diet low in branched chain amino acids.

However, a milder form of the disease called intermittent branched chain ketonuria responds to high doses of thiamine. This is because the decarboxylation of the BCKA requires thiamine.

Liver transplantation has been successfully tried in some cases of MSUD.

Homocystinuria type I

The homocystinurias are a group of disorders involving defects in the metabolism of homocysteine. These autosomal-recessive diseases are characterized by high plasma and urinary levels of homocysteine and methionine and low levels of cysteine.

Homocystinuria type I is the classical form of homocystinuria.

lt is due to a defect in the enzyme cystathionine synthase and results in the accumulation of homocystine.

Plasma level of homocystine increases and excreted in urine. In some cases, S-adenosyl methionine is also excreted.

Clinical features

Mental retardation
Hepatomegaly
Atherosclerosis
Ectopialentis (dislocation of lens of the eye)
thrombosis
osteoporosis
Most of the patients show abnormal EEG.

Treatment

Two forms of type I homocystinurias are known, one of them can be corrected with vitamin B6 supplementation (B6 responsive) while the other does not respond to B6. The treatment includes consumption of diet low in methionine and high in cystine

The other homocvstinurias are associated with enzyme defects in the conversion of homocysteine to methionine by remethylation.

Homocystinuria II

Homocystinuria II is autosomal recessive.

In homocystinuria II, there is deficiency of the enzyme N5-methyl-Tetrahydrofolate-homocysteine methyl transferase.

Clinical features

Mental retardation

No ectopialentis or thrombotic episodes seen.

There is increased plasma level of homocysteine.

Homocysteine is excreted in urine.

Treatment

Responds to folic acid administration.

Homocystinuria III

Homocystinuria III is autosomal recessive.

In homocystinuria III, there is deficiency of the enzyme N5, N10-methylene tetrahydrofolate reductase.

Clinical features

Mental retardation

No ectopialentis or thrombotic episodes seen.

There is increased plasma level of homocysteine.

Homocystine is excreted in urine.

Treatment

Responds to folic acid administration.

Creatine biosynthesis

Three amino acids are required in the biosynthesis of creatine:

Glycine

Arginine

Methionine (as S-adenosylmethionine)

Site of biosynthesis:

Step 1: Kidneys

Step 2: Liver

Distribution of body creatine

From liver, creatine is transported to other tissues

98% of creatine are present in skeletal and heart muscles

In Muscle, it gets converted to the high energy source creatine phosphate (phosphocreatine)

Creatine phosphate is a high-energy phosphate compound

It acts as a storage form of energy in the muscle

Provides a small but, ready source of energy during first few minutes of intense muscular contraction

The amount of creatine phosphate in the body is proportional to the muscle mass

Creatine degradation

Creatine and creatine phosphate spontaneously form creatinine as an end product

Creatinine is excreted in the urine

Serum creatinine is a sensitive indicator of kidney disease (Kidney function test)

Serum creatinine increases with the impairment of kidney function

Urinary creatinine

A typical male excretes about 15 mmol of creatinine per day

A decrease in muscle mass due to muscular dystrophy or paralysis leads to decreased level of creatinine in urine

The amount of creatinine in urine is used as an indicator for the proper collection of 24 hours urine sample.

The normal concentration of creatine and creatinine in human serum and urine are as follows:

Serum:	Creatine - 0.2 ï 0.6mg/dl	Creatinine ï 0.6 ï 1mg/dl
Urine:	Creatine ï 0 ï 50mg/day	Creatinine ï 1 ï 2g/day

Estimation of serum creatinine (along with blood urea) is used as a diagnostic test to assess kidney function.

Serum creatinine concentration is not influenced by endogenous and exogenous factors, as is the case with urea.

Hence, some workers consider serum creatinine as a more reliable indicator of renal function.

lncreased output of creatine in urine is referred to as creatinuria. Creatinuria is observed in muscular dystrophy, diabetes mellitus, hyperthyroidism, starvation etc.

Introduction to Purine and Pyrimidine Metabolism

Purines and pyrimidines are heterocyclic structures that contain carbon and nitrogen. They are referred to as organic bases. Purines: adenine and guanine. Pyrimidines: cytosine, uracil and thymine. Purines and pyrimidines are synthesized from amphibolic intermediates in human tissues. Ingested nucleic acids and nucleotides are degraded in the intestinal tract to mononucleotides, which may be absorbed or converted to purine and pyrimidine bases. The purine bases are then oxidized to uric acid, which may be absorbed and excreted in the urine

Nucleosides

Nucleosides are derivatives of organic bases; purine and pyrimidine that have a sugar linked to the nitrogen atom of a purine or pyrimidine. D-ribose is the sugar moiety in ribonucleosides while 2-deoxy-D-ribose is the sugar moiety in deoxyribonucleosides. These sugars are linked to the heterocycle by a ï N-glycosidic bond, usually to the N-1 of a pyrimidine or to N-9 of a purine.

Nucleotides

Nucleotides are phosphorylated nucleosides. The phosphoryl group is esterified to a hydroxyl group of the sugar. Examples are adenine monophosphate, guanine monophosphate**.** All forms of life excluding parasitic protozoa synthesize purine and pyrimidine nucleotides.

Catabolism of Purines

Adenosine and guanosine are catabolized into uric acid. Adenosine is first converted to inosine by adenosine deaminase.

Abnormalities of Purine Catabolism

Lesch-Nyhan Syndrome: An overproduction of uric acid (hyperuricaemia). It is caused by a deficiency in the activity of hypoxanthine-guanine phosphoribosyl transferase.

Von Gierke Disease: It is caused by glucose-6-phosphatase deficiency. This results in purine overproduction and hyperuricaemia.

Hypouricaemia: this is caused by xanthine oxidase deficiency as a result of genetic defect or to severe liver damage. This often result in increased excretion of hypoxanthine and xanthine

Adenosine Deaminase deficiency: this is associated with immunodeficiency in which both thymus-derived lymphocytes (T cells) and bone-marrow-derived lymphocytes (B cells) are sparse and dysfunctional. Patients therefore suffer from severe immunodeficiency- probne to infections

Purine nucleoside phosphorylase deficiency: this is associated with severe deficiency of T cells but apparently normal B cell function. This results in ummune dysfunctions

Catabolism of Pyrimidines

The end products of pyrimidine catabolism are water soluble: NH_3, CO_2, alanine and aminoisobutyrate. Excretion of aminoisobutyrate increases leukaemia.

Abnormalities of Pyrimidine Catabolism

Disorders of alanine and aminoisobutyrate metabolism arise from defects in the enzymes of pyrimidine catabolism.

Hydroxybutyric aciduria: caused by the deficiency of dihydropyrimidine dehydrogenase

Uraciluria-thyminuria: There is presence of uracil and thymine in the urine

Synthetic Analogues of Nucleotide

Synthetic analogs of purines, pyrimidines, nucleosides and nucleotides have various applications in medicine. They are used in the management of cancer. Examples are 5-fluoro- or 5-iodouracil, 3-deoxyuridine, 6-thioguanine and 6-mercaptopurine, 5- or 6- azauridine, 5- or 6-azacytidine, and 8-azaguanine. They are incorporated into DNA prior to cell division. They are also used in the treatment of Gout. Allopurinol (an analog of purine) is used in treatment of hyperuricemia and gout. It inhibits purine biosynthesis and xanthine oxidase activity. They are also used in organ transplantation. Azathioprine is used in organ transplantation. It is catabolized to 6-mercaptopurine. It suppresses immunologic rejection

The DNA

Intended Learning Outcomes

At the end of the lecture, students should be able to

1. Explain the chemical composition of the DNA
2. Relate the DNA, gene and chromosome
3. Discuss the denaturation and renaturation of DNA

The DNA consists of the organic bases i.e. adenine (A), guanine (G), cytosine (C) and thymine (T). These bases are held in linear array by phosphodiester bonds through the 3ô and 5ô positions of adjacent deoxyribose moieties. The DNA is organized into two strands by the pairing of bases A to T and G to C. The two complementary chains are held by hydrogen bonds between the base portions of the nucleotide. The chemical polarity of DNA strand is maintained by the unique way the nucleotide subunits are linked. There are 3.2×10^9 base pairs of DNA in humans organized into 23 chromosomes.

The DNA carries the genes. A gene is a segment of the DNA that encodes the information required to produce a functional biological product. The complexity of an organism correlates with the number of genes in its genome. For example, the total number of genes ranges from less than 500 in a bacterium to about 30,000 in humans.

Chromosomes

The nucleic acid molecules are the repository of an organismôs genetic information. They are the largest molecules in a cell and may contain thousands of genes as well as considerable tracts of intergenic DNA. Chromosomes are linked with proteins involved in gene expression, DNA replication and repair

In eukaryotic cells, each chromosome consists of a single, very long linear DNA molecule associated with proteins that fold and pack the DNA into a more compact structure. The packaging of DNA generates a series of coils and loops that provide higher levels of organization that prevents the DNA from becoming an unmanageable tangle.

A DNA molecule performs other functions in addition to carrying genes; it must be able to replicate, the replicated copies must be separated and partitioned reliably into daughter cells at each cell division. These processes occur through an ordered series of stages, known collectively as the cell cycle.

There are two important stages of the cell cycle; interphase and mitosis.

Interphase: This is the period between one mitotic (M) phase and the next. It encompasses the remaining three phases of the cell cycle; synthesis (S) phase, gap (G_1) phase and G_2 phase. The following events occur in the interphase; duplication of chromosomes and protein synthesis.

Mitosis: chromosomes are distributed to the two daughter nuclei in this stage of the cell cycle.

DNA Replication

This is a process by which a DNA molecule is duplicated. At each cell division, a cell must accurately copy its genome. DNA replication produces two complete double strands from the original DNA molecule. Each new DNA helix is identical in nucleotide sequence to the parental DNA double helix. DNA replication is initiated by DNA polymerase.

Denaturation of DNA

This is the process of separating the double-stranded structure of the DNA into two component strands in a solution. This is done by increasing the temperature and decreasing the salt concentration. This separates the two stacks of bases. The bases unstack while still connected in the polymer by the phosphodiester backbone. Denaturation of the DNA increases the optical absorbance of the purine and pyrimidine bases. This is called hyperchromicity of denaturation. DNA rich in G-C pairs (with 3 hydrogen bonds) melts at a higher temperature than that rich in A-T pairs (with 2 hydrogen bonds)

Renaturation of DNA

This is a process whereby separated DNA strands re-associate under normal physiologic temperature and salt conditions**.** The rate of re-association depends on the concentration of the complementary strands. Example: the re-association of the two complementary DNA strands of a chromosome after transcription.

Topic: The RNA

Intended Learning Outcomes

At the end of the lecture, students should be able to

1. Discuss the classes of RNA
2. Distinguish between DNA and RNA

Introduction

The RNA consists of ribose sugar linked with organic bases and phosphoryl group. The bases in RNA includes; adenine (A), uracil (U), cytosine (C) and guanine (G). Thymine is not found in RNA. Adenine pairs with uracil, cytosine pairs with guanine. It is synthesized from a DNA template by a process called transcription. It entails the transfer of information from DNA where it is stored into RNA which can be transported and interpreted. RNA synthesis is catalysed by RNA polymerase.

Ribonucleotide sequence in RNA molecule is complementary to the deoxyribonucleotide sequence in a strand of DNA molecule

Template Strand: the strand that is transcribed into RNA molecule.

Non template (coding) strand: the non template strand.

Transcription unit: the region of DNA that includes the signals for transcription

Classes of RNA

Eucaryotic cells have four major classes of RNA

1. Messenger RNA (mRNA)
2. Ribosomal RNA (rRNA)
3. Transfer RNA (tRNA)
4. Small nuclear RNA (snRNA) and micro nuclear RNA (miRNA)

mRNA, rRNA and tRNA are involved in protein synthesis

Messenger RNA (mRNA)

This is the most heterogenous class of RNA in; abundance (likely varies over 10^4 fold range), size and stability. Every member of this class of RNA functions as messenger conveying the information in a gene to the protein synthesizing machinery. It serves as a template on which specific sequence of amino acids is polymerized to form a specific protein molecule.

Transfer RNA (tRNA)

Transfer RNA serves as an adapter for the translation of information in the sequence of mRNA into specific amino acids. It varies between 79 and 95 nucleotides in length. There are about 20 species of tRNA in every cell. The nucleotide sequence of tRNA molecules allows extensive folding and intra-strand complementarity to generate a secondary structure

Ribosomal RNA (rRNA)

This class of RNA is located in the ribosome. It is made from pre-ribosomal RNA. Ribosomal RNA is involved in protein synthesis. It represents about 70% of cellular RNA.

Short Nuclear (snRNA) and Micro Nuclear (miRNA)

They are important in gene regulation and mRNA splicing

Differences between DNA and RNA

1. DNA is double-stranded, RNA is single-stranded
2. There is a difference in the pyrimidine component of DNA and RNA. DNA contains adenine, thymine, guanosine and cytosine RNA contains adenine, uracil, guanosine and cytosine.
3. The sugar moiety of RNA is ribose. The sugar moiety of DNA is deoxyribose.
4. Since the RNA molecule is a single strand complementary to only one of the two strands of a gene, its guanine content does not necessarily equal its cytosine content, nor does its adenine content necessarily equal its uracil content
5. RNA can be hydrolyzed by alkali to 2',3' cyclic diesters of the mononucleotides, compounds that cannot be formed from alkali-treated DNA because of the absence of a 2'-hydroxyl group. The alkali lability of RNA is useful both diagnostically and analytically

Topic: Metabolism of one carbon units

Intended Learning Outcomes

At the end of the lecture, students should be able to

1. Discuss the absorption and bioavailability of folic acid
2. Discuss the catabolism and excretion of folic acid
3. Reactions involving folate

Folic Acid

Folic acid serves as a carrier of one carbon groups in many metabolic reactions. It is required for the biosynthesis of compounds such as purines, serine, glycine, choline and deoxythymidine monophosphate (dTMP). Folic acid or folate function as a coenzyme. They are derived from pteroic acid to which one or more molecules of glutamic acid are attached.

Multiple forms of folic acid occur with substitutions of functional groups such as methyl, formyl, methylene, hydroxymethyl etc at nitrogen atoms in the pteroic acid residue usually N^5 or bridging N^5 and N^{10}. 5-methyltetrahydrofolate is the principal form of folic acid in the human serum and other body fluids.

Dietary Sources

The principal food sources of folate are liver, spinach and other dark green leafy vegetables, legumes such as kidney and lima beans, orange juice etc

Absorption and Bioavailability

Folate is absorbed from dietary sources mainly as reduced methyl and formyl-tetrahydropteroylpolyglutamates. The bioavailability of folate from food sources is variable and depends on factors such as;

1. Incomplete release from plant cellular structure,
2. Entrapment in food matrix during digestion,

3. Inhibition of deglutamation by other dietary constituents Possibly the degree of polyglutamation.

The bioavailability of supplemental folic acid is greater than that of food folate. It can be as high as 100% for folic acid supplements taken on an empty stomach compared with about 50% for food folates. Polyglutamate forms of folate present in food are first converted to monoglutamates by pteroylpolyglutamate hydrolase in the intestinal mucosa. Thereafter, most of the folate is reduced and methylated and enters the circulation as 5-methyltetrahydrofolate (5-MTHF), circulating loosely bound to albumin or to folate-binding protein. Once within the cell, 5-MTHF is demethylated and converted to the polyglutamyl form by folypolyglutamate synthase which helps to retain folate within the cell, this is because it is unable to cross cell membranes.

The polyglutamates are reconverted to monoglutamates by polyglutamate hydrolase before they are released into the circulation. Folic acid and vitamin B12 metabolism are linked by the reaction that transfers a methyl group from 5-MTHF to cobalamin. In cases of cobalamin deficiency, folate is trapped as 5-MTHF and is metabolically dead.

It cannot be recycled as tetrahydrofolate (THF) back into the folate pool to serve as the main one-carbon unit acceptor for many biochemical reactions. Eventually, cellular depletion of MTHF ensues, causing a reduction in thymidylic acid synthesis, which in turn results in megaloblastic anaemia and neuropathies.

Excretion

Protein-free plasma folate is filtered at the glomerulus and most is reabsorbed by the proximal renal tubules. Therefore, intact urinary folate is only a small percentage of intake. Folate is predominantly excreted by catabolism following cleavage of the C9-N10 bond to produce p-aminobenzoylpolyglutamates which are then hydrolyzed to monoglutamates and N-acetylated before excretion.

Functions of Folate

1. Folate coenzymes as well as coenzymes derived from vitamins B12, B6 and B2 are essential for one carbon metabolism.
2. Biochemically, a carbon unit from serine or glycine is transferred to tetrahydrofolate (THF) which is then used in the synthesis of thymidine which is incorporated into DNA oxidized to formyl-THF for use in the synthesis of purines precursors of RNA and DNA or reduced to methyl-THF which is necessary for the methylation of homocysteine to methionine.
3. Much of this methionine is converted to S-adenosylmethionine, a universal donor of methyl groups to DNA, RNA, hormones, neurotransmitters, membrane lipids and proteins.

Anaemia

Anaemia is a condition in which the blood has a lower than normal concentration of haemoglobin, which results in a reduced ability to transport oxygen.

Nutritional anaemia

This is caused by inadequate intake of one or more essential nutrients. It can be classified according to the size of the red blood cells (RBCs) or mean corpuscular volume (MCV) observed in the individual.

Nutritional anaemia can be divided into microcytic and macrocytic anaemias

Microcytic anaemia: This is caused by lack of iron. It is the most common form of nutritional anaemia. The MCV is below normal.

Macrocytic anaemia: It results from a deficiency in folic acid, or vitamin B12. The MCV is above normal. Macrocytic anaemias are commonly called megaloblastic because a deficiency of either vitamin (or both) causes accumulation of large, immature RBC precursors, known as megaloblasts, in the bone marrow and the blood. The normal mean corpuscular volume (MCV) for people older than age 18 is between 80 and 100 εm^3

Folate and Anaemia

Inadequate serum levels of folate can be caused by increased demand (for example, pregnancy and lactation), poor absorption caused by pathology of the small intestine, alcoholism, treatment with drugs that are dihydrofolate reductase inhibitors. E.g. methotrexate.

Folate and neural tube defect

Folic acid supplementation before conception and during the first trimester has been shown to significantly reduce neural tube defects (NTDs). Example: Spina bifida

All women of childbearing age are advised to consume 0.4 mg/day of folic acid to reduce the risk of having a pregnancy affected by NTDs and ten times that amount if a previous pregnancy was affected. Adequate folate nutrition must occur at the time of conception because critical folate-dependent development occurs in the first weeks of foetal life. This is a time when many women are not yet aware of their pregnancy.

Topic: Metabolism of Inorganic Nitrogen

Intended Learning Outcome

At the end of this lecture, students should be able to

1. Explain the metabolism of inorganic nitrogen in living systems

Amino acid catabolism is part of the larger process of the metabolism of nitrogen containing molecules. Nitrogen enters the body in a variety of compounds present in food, the most important being amino acids contained in dietary protein. Nitrogen leaves the body as urea, ammonia, and other products derived from amino acid metabolism. The role of body proteins in these transformations involves two important concepts: The amino acid pool and protein turnover.

The amino acid pool

Free amino acids are present throughout the body, such as in cells, blood, extracellular fluids. These amino acids can be said to belong to a single entity called the amino acid pool. This pool is supplied by three sources:

1. Amino acids provided by the degradation of endogenous (body) proteins, most of which are reutilized;
2. Amino acids derived from exogenous (dietary) protein
3. Non essential amino acids synthesized from simple intermediates of metabolism.

Conversely, the amino pool is depleted by three routes:

1. Synthesis of body protein.
2. Consumption of amino acids as precursors of essential nitrogen-containing small molecules.
3. Conversion of amino acids to glucose, glycogen, fatty acids and ketone bodies or oxidation to CO_2 + H_2O. Although the amino acid pool is small (comprising about 90ï 100 g of amino acids) in comparison with the amount of protein in the body (about 12 kg in a 70 kg man).

Protein turnover

The total amount of protein in the body of a healthy adult remains constant because the rate of protein synthesis is just sufficient to replace the protein that is degraded. This process, called protein turnover, leads to the hydrolysis and re-synthesis of between 300 and 400 g of body protein each day.

The rate of protein turnover varies widely for individual proteins.

Short-lived proteins (for example, many regulatory proteins and mis-folded proteins) are rapidly degraded, They have half-lives measured in minutes or hours. Long-lived proteins, with half-lives of days to weeks, constitute the majority of proteins in the cell. Structural proteins, such as collagen, are metabolically stable and have half-lives measured in months or years

Protein degradation:

There are two major enzyme systems responsible for degrading proteins:

1. Ubiquitinï proteasome proteolytic pathway
2. Chemical signals for protein degradation

Ubiquitinï proteasome proteolytic pathway

Proteins selected for degradation by the cytosolic ubiquitin-proteasome system are first modified by the covalent attachment of ubiquitin (Ub), a small, globular, nonenzymic protein that is highly conserved across eukaryotic species. Ubiquitination of the target substrate occurs through isopeptide linkage of the Ŭ-carboxyl group of the C terminal glycine of Ub to the Ů-amino group of a lysine on the protein substrate. This occurs by a three-step, enzyme-catalyzed, ATP-dependent process. Enzyme 1 (E1, or activating enzyme) activates Ub, which is then transferred to E2 (conjugating enzyme). E3 (a ligase) identifies the protein to be degraded and interacts with E2-Ub.

The consecutive addition of four or more Ub molecules to the target protein generates a polyubiquitin chain. Proteins tagged with Ub are recognized by a large, barrel-shaped, macromolecular, proteolytic complex called a proteasome.

The proteasome unfolds, deubiquitinates, and cuts the target protein into fragments that are then further degraded by cytosolic proteases to amino acids, which enter the amino acid pool. Ub is recycled. It is noteworthy that the selective degradation of proteins by the ubiquitinproteosome complex unlike simple hydrolysis by proteolytic enzymes) requires energy in the form of ATP.

Chemical signals for protein degradation

Because proteins have different half-lives, it is clear that protein degradation cannot be random but, rather, is influenced by some structural aspect of the protein. For example, some proteins that have been chemically altered by oxidation or tagged with ubiquitin are preferentially degraded.

The half-life of a protein is also influenced by the amino (N)-terminal residue. For example, proteins that have serine as the N-terminal amino acid are long-lived, with a half-life of more than 20 hours, whereas those with aspartate at their N-terminus have a half-life of only 3 minutes. Additionally, proteins rich in sequences containing proline, glutamate, serine, and threonine are rapidly degraded and, therefore, have short half-lives.

Digestion of protein

On entering the small intestine, large polypeptides produced in the stomach by the action of pepsin are further cleaved to oligopeptides and amino acids by a group of pancreatic proteases that include both endopeptidases (cleave within) and exopeptidases (cut at an end).

Abnormalities in protein digestion

Digestion and absorption of fat and protein are incomplete in individuals with a deficiency in pancreatic secretion which could be due to

1. Chronic pancreatitis
2. Cystic fibrosis
3. Surgical removal of the pancreas

This results in the abnormal appearance of lipids in the faeces (a condition called steatorrhea) as well as undigested protein.

The Urea Cycle

Urea is the major disposal form of amino groups derived from amino acids. It constitutes about 90% of the nitrogen-containing components of urine. A nitrogen of the urea molecule is supplied by free ammonia and the other nitrogen by aspartate. Glutamate is the immediate precursor of both ammonia (through oxidative deamination by glutamate dehydrogenase) and aspartate nitrogen (through transamination of oxaloacetate by AST). The carbon and oxygen of urea are obtained from CO_2 (as $HCO_3^{\ddot{I}}$). Urea is produced by the liver and then is transported in the blood to the kidneys for excretion in the urine.

Fate of urea:

Urea diffuses from the liver and is transported in the blood to the kidneys, where it is filtered and excreted in the urine. A portion of the urea diffuses from the blood into the intestine and is cleaved to CO_2 and NH_3 by bacterial urease. This ammonia is partly lost in the faeces and is partly reabsorbed into the blood. In patients with kidney failure, plasma urea levels are elevated, promoting a greater transfer of urea from blood into the gut. The intestinal action of urease on this urea becomes a clinically important source of ammonia, contributing to the hyperammonemia often seen in these patients. Oral administration of antibiotics reduces the number of intestinal bacteria responsible for this NH_3 Production

Summary and Conclusion

This course has informed students on genome organization, organic bases (purines and pyrimidines), nucleosides and nucleotides as well as abnormalities in their metabolism. The metabolisms of nucleic acids, one carbon units and inorganic nitrogen were also discussed.

Interactions and Questions

1. Discuss the metabolism of protein
2. Discuss the urea cycle
3. What are inborn errors of metabolism
4. Draw the structure of Thymidine
5. Discuss the classes of RNA
6. Distinguish between DNA and RNA

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