Course Title: Medical Biochemistry II

RAD 2 nd , Sec A

Lab Assignment

Student Name: Afsha Farid

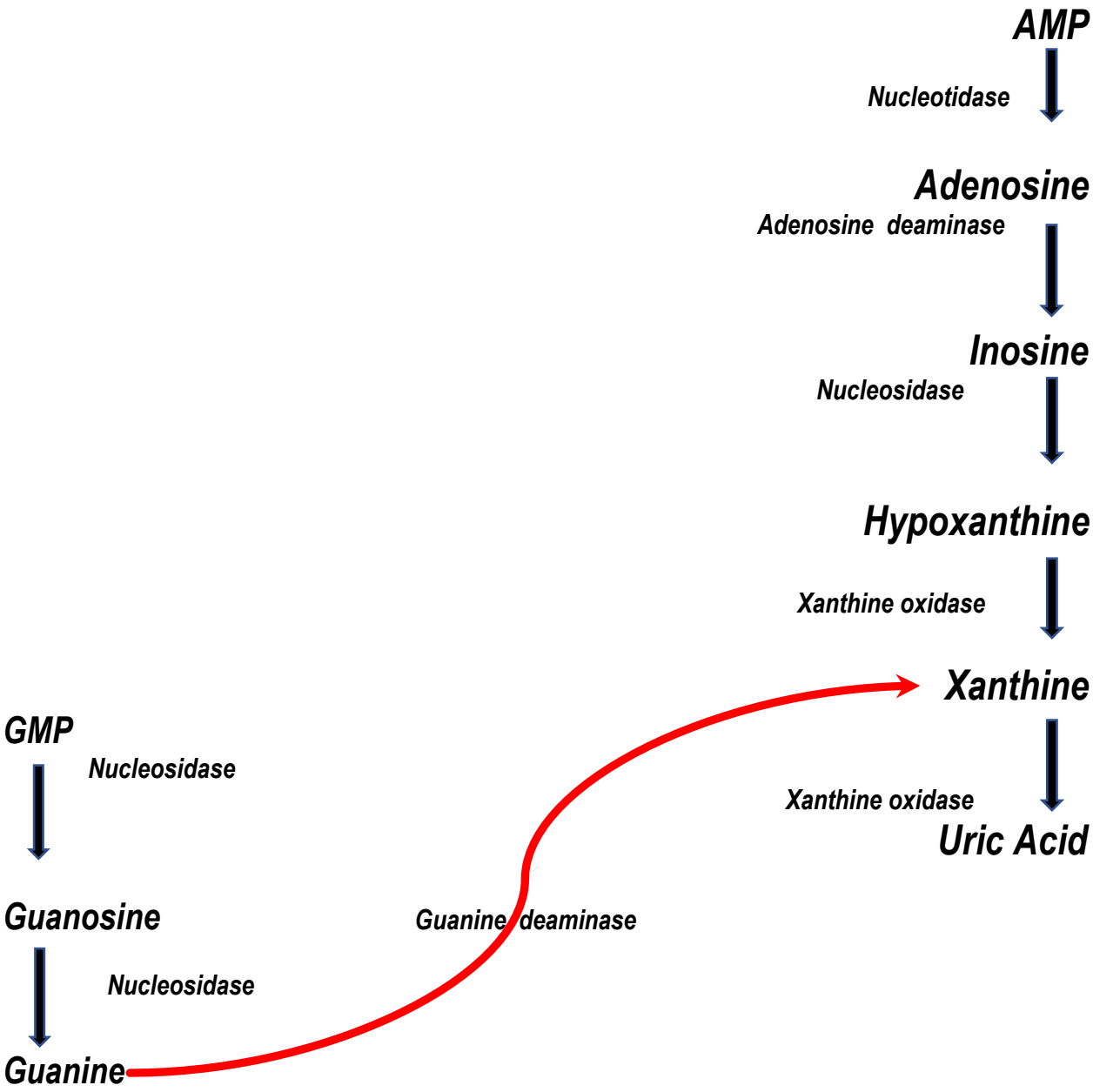
Student ID: 15918

Note: Avoid copy paste material, as it may deduct your marks.

Q1. Explain the process of Uric Acid Formation.

URIC ACID

Introduction: *Uric acid production and metabolism are complex processes involving various factors that control hepatic production, as well as renal and gut excretion of this compound. Uric acid is the end product of an exogenous group of purines and endogenous purine metabolism. Uric acid is a heterocyclic organic compound $C_5H_4N_4O_3$ and has a molecular weight of 168 Da. In the conversion of two nucleic acids (Adenine and Guanine), enzymes are involved to produce uric acid. Uric acid crystal formation increases when urate concentration increases in blood. The quantity of uric acid in human blood is 1.5 to 6.0mg/dL in women and in men is 2.5 to 7.0 mg/dL.*



Formation of uric acid :

A summary of the steps in the production of uric acid and genetic diseases related with deficiencies of specific degradative enzymes.

STEPS

[1] An amino group is removed from AMP to produce IMP by AMP deaminase or from adenosine to produce inosine (hypoxanthine-ribose) by adenosine deaminase.

[2] Inosine mono-PO4 (IMP) and Guanosine mono-PO4 (GMP) are transformed into their nucleoside forms (inosine and guanosine) by the action of 5 -nucleotidase.

[3] Purine nucleoside phosphorylase transforms inosine and guanosine into their respective purine bases, hypoxanthine and guanine. [Note: A mutase interconverts ribose 1- and ribose 5-phosphate.]

[4] xanthine is formed when guanine is deaminated.

[5] Hypoxanthine is oxidized by xanthine oxidase to xanthine, which is further oxidized by xanthine oxidase to uric acid, the final product of human purine degradation. Uric acid is excreted primarily in the urine.

Diseases associated with purine degradation

*1. **Gout:** Gout is a disorder initiated by high levels of uric acid (the end product of purine catabolism) in blood (hyperuricemia), as a result of either the **overproduction** or **underexcretion** of uric acid.*

- *The hyperuricemia can lead to the deposition of monosodium urate (MSU) crystals in the **joints** and an inflammatory response to the crystals, causing first acute and then progressing to chronic **gouty arthritis.***
- *Nodular masses of MSU **crystals** (tophi) may be deposited in the **soft tissues**, resulting in chronic tophaceous gout.*
- *Formation of **uric acid stones** in the **kidney (urolithiasis)** may also be seen.*
- *The definitive diagnosis of gout requires ambition and examination of **synovial fluid** from an affected **joint** (or material from a tophus) using polarized light microscopy to confirm the presence of **needle shaped MSU crystals***

[Note: Hyperuricemia, while necessary, is not sufficient to cause gout, but gout is always preceded by hyperuricemia. Hyperuricemia is typically asymptomatic but may be indicative of comorbid conditions such as hypertension.]

a. Underexcretion of uric acid: *In* ***over 90%*** *of individuals, hyperuricemia is caused by underexcretion of uric acid. Underexcretion can be primary, due to as yet-unidentified inherent excretory defects, or secondary to known disease processes that assume how the kidney handles urate (for example, in lactic acidosis, lactate increases renal urate reabsorption, thereby decreasing its excretion) and to environmental factors such as the use of drugs (for example, thiazide diuretics) or exposure to lead (saturnine gout).*

b. Overproduction of uric acid: *A less common cause of hyperuricemia is from the overproduction of uric acid. Primary hyperuricemia is, for the most part, idiopathic (having no known cause). However, several identified mutations in the gene for X-linked PRPP synthetase result in the enzyme having an increased maximal velocity (Vmax) for the production of PRPP, a lower Km for ribose 5-phosphate, or a decreased sensitivity to purine nucleotides, its allosteric inhibitors. In each case, increased availability of PRPP increases purine production, resulting in elevated levels of plasma uric acid.*

Lesch-Nyhan syndrome *also causes hyperuricemia as a result of the decreased salvage of hypoxanthine and guanine and the following increased availability of PRPP. Secondary hyperuricemia is typically the concern of increased availability of purines.*

Treatment of gout: *Acute attacks of gout are pickled with anti-inflammatory agents. Colchicine; steroidal drugs, such as prednisone; and nonsteroidal drugs, such as indomethacin, are used. Allopurinol inhibits xanthine oxidase, resulting in accumulation of hypoxanthine and xanthine, compounds more soluble than uric acid.*

2. Adenosine deaminase deficiency:

Adenosine deaminase (ADA) is expressed in a variety of tissues, but, in humans, lymphocytes have the highest activity of this cytoplasmic enzyme. A deficiency of ADA results in an accumulation of adenosine, which is converted to its ribonucleotide or deoxyribonucleotide forms by cellular kinases. As dATP levels rise, ribonucleotide reductase is inhibited, thereby preventing the production of all deoxyribose-containing nucleotides .Consequently, cells cannot make DNA and divide. In its most severe form, this autosomal-recessive disorder causes a type of severe merged immunodeficiency disease (SCID), involving a decrease in ***T cells, B cells, and natural killer cells.***

Treatments:

1. bone marrow transplantation,
2. enzyme replacement therapy,
3. gene therapy.

Without appropriate treatment, children with this disorder usually die from infection by age 2.

Q2. Discuss all the protein complexes used in Electron transport chain.

ANS: Electron transport chain:

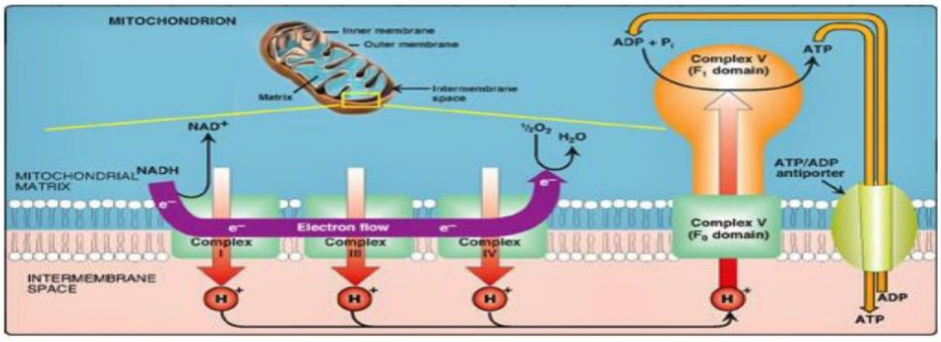
DEFINITION: *The electron transport chain is a cluster of proteins that transfer electrons through a membrane within a mitochondria to form a gradient of protons that drives the creation of ATP.*

PROTEIN COMPLEXES:

- *Where on the inner membrane there are four attach protein complexes.*
- *These protein complexes process the ETC.*
- *The coenzyme Q is present between complex 1 and 2, the molecules of cytochrome is present between 3 and 4 complex protein.*
- *In the cytochrome molecule there are four arranged molecules like cyt b, cyt c, cyt a, and cyt a3 arranged in a specific pattern.*
- *Apart from these protein complexes and enzyme ATP synthase is also present.*
- *ATP synthase help in the production of ATP.*
- *These membranes bound electron carriers passed on the electrons to the other electron carriers until they are finally given to O2 and produce water.*

Reactions of the electron transport chain:

With the exception of coenzyme Q, which is a lipid-soluble quinone, all members of this chain are proteins. These may function as enzymes as is the case with the flavin containing dehydrogenases, may contain iron as part of an iron-sulfur center, may contain iron as part of the porphyrin prosthetic group of heme as in the cytochromes, or may contain copper as does the cytochrome a + a 3 complex.



COMPLEX 1: NADH dehydrogenase:

- *The free proton plus the hydride ion carried by NADH are transferred to NADH dehydrogenase, a protein complex (Complex I) embedded in the inner mitochondrial membrane.*
- *Complex I has a tightly bound molecule of flavin mononucleotide (FMN), a coenzyme structurally related to FAD that accepts the two hydrogen atoms (2e – + 2H+), becoming FMNH2 .*
- *NADH dehydrogenase also contains peptide subunits with iron-sulfur centers.*
- *At Complex I, electrons move from NADH to FMN to the iron of the iron-sulfur centers and then to coenzyme Q.*

- *As electrons flow, they lose energy.*
- *This energy is used to pump protons across the inner mitochondrial membrane, from the matrix to the intermembrane space.*

COMPLEX 2: Succinate dehydrogenase:

- *Now, the most energetic molecule FADH2 get oxidize and reduce the complex 2 molecule by giving its electrons and convert itself into FADH form.*
- *The electrons from complex 2 transfer to coenzyme Q and it transfer the electrons to complex 3 and finally reach to the complex 4.*
- *No H+ ions are transported to the inter membrane space in this process.*

COMPLEX 3: Cytochromes bc1:

- *The Coenzyme Q itself get oxidized by giving electrons to the complex 3 molecule and reduce it.*
- *This process releases energy which transfer the H+ ion from matrix to the inter membranal space.*
- *The complex 3 transfer its electrons to the cytochrome and itself get oxidize.*
- *This redox rxn do not produce as much energy, so that the H+ ion do not transfer to the inter membranal space.*

COMPLEX 4: Cytochromes a + a3:

- *This cytochrome complex (Complex IV) is the only electron carrier in which the heme iron has an available coordination site that can react directly with O2 and so also is called cytochrome oxidase.*

- *At Complex IV, the transported electrons, O2 , and free protons are brought together, and O 2 is reduced to water [Note Four electrons are required to reduce one molecule of O 2 to two molecules of water.]*

- *Cytochrome oxidase contains copper (Cu) atoms that are required for this complicated reaction to occur.*

- *Electrons move from Cu A to cytochrome a to cytochrome a 3 (in association with CuB) to O2 .*

COMPLEX 5: ATP synthase:

- *The multi subunit enzyme ATP synthase synthesizes ATP using the energy of the proton gradient.*

- *It contains a domain (Fo) that spans the inner mitochondrial membrane, and an extra membranous domain (F1) that appears as a sphere that protrudes into the mitochondrial matrix.*

- *The chemiosmotic hypothesis proposes that after protons have been pumped to the cytosolic side of the inner mitochondrial membrane, they reenter the matrix by passing through a proton channel in the Fo domain, driving rotation of the c ring of Fo and, at the same time, dissipating the pH and electrical gradients.*

- *Fo rotation causes conformational changes in the beta subunits of the F1 domain that allow them to bind ADP + P i, phosphorylate ADP to ATP, and release ATP.*