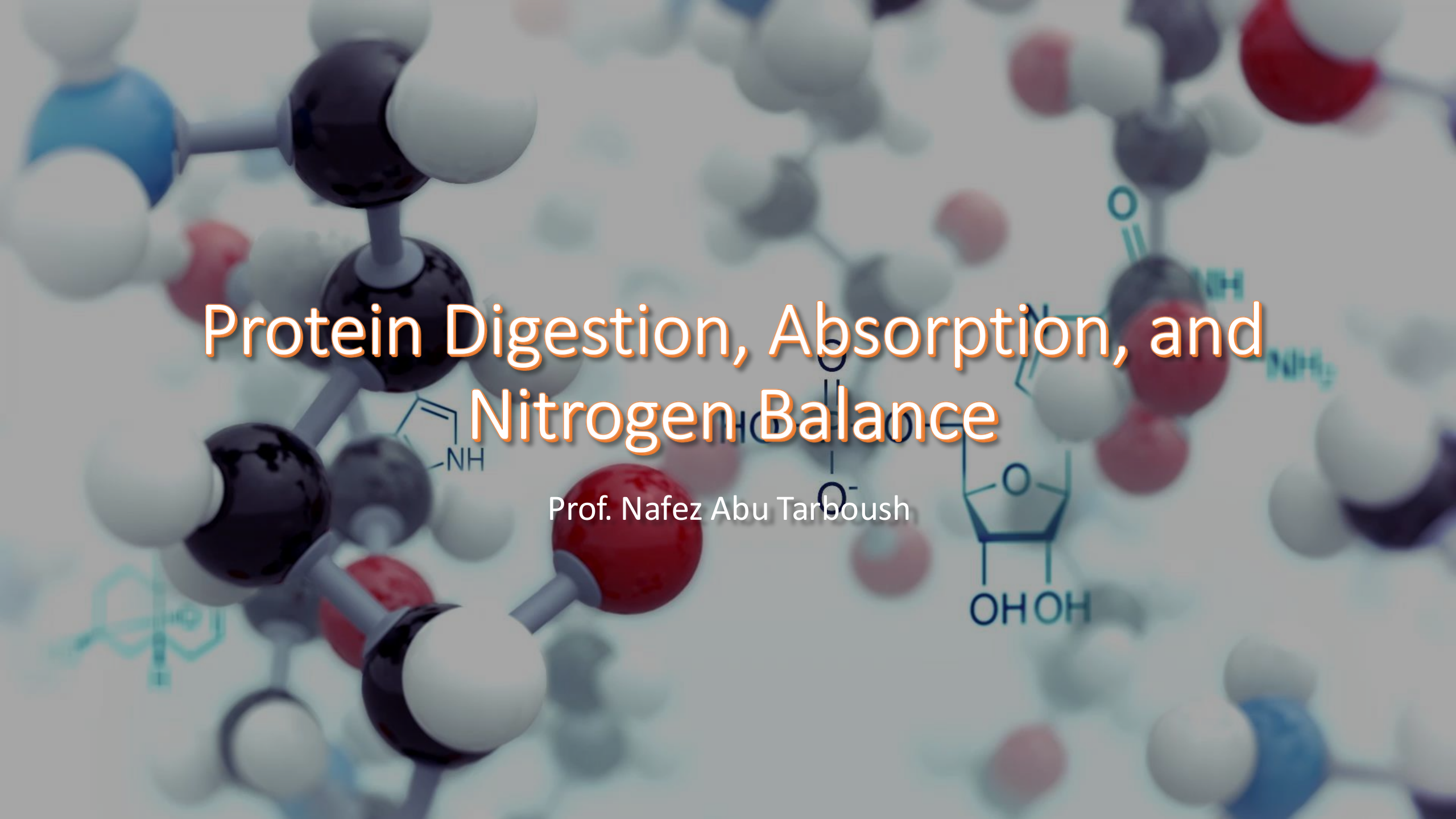
بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Metabolism | Final 14

Protein digestion, Absorption, & nitrogen balance pt.1

Written & Reviewed by : **NST**

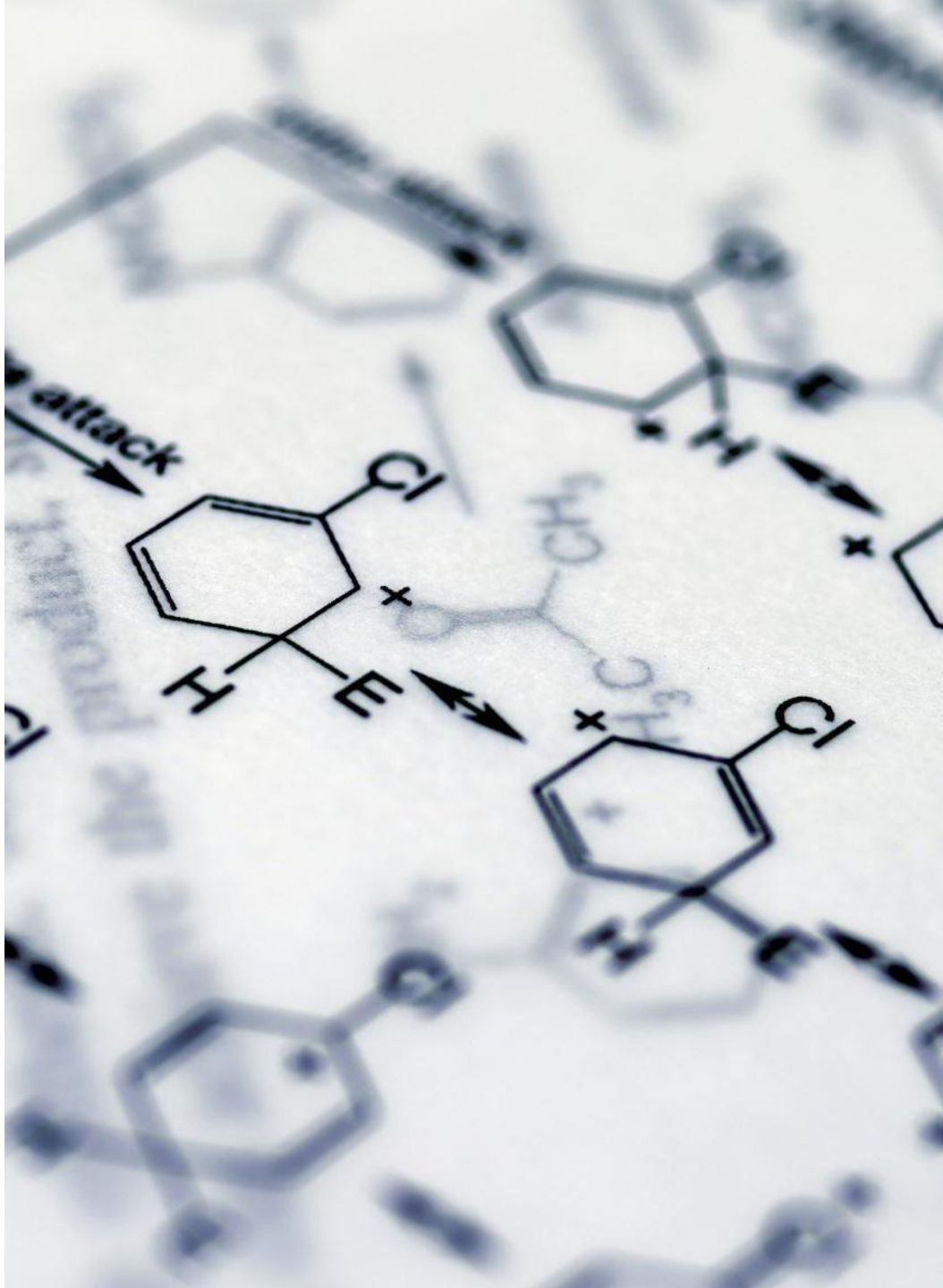


Protein Digestion, Absorption, and Nitrogen Balance

Prof. Nafez Abu Tarboush

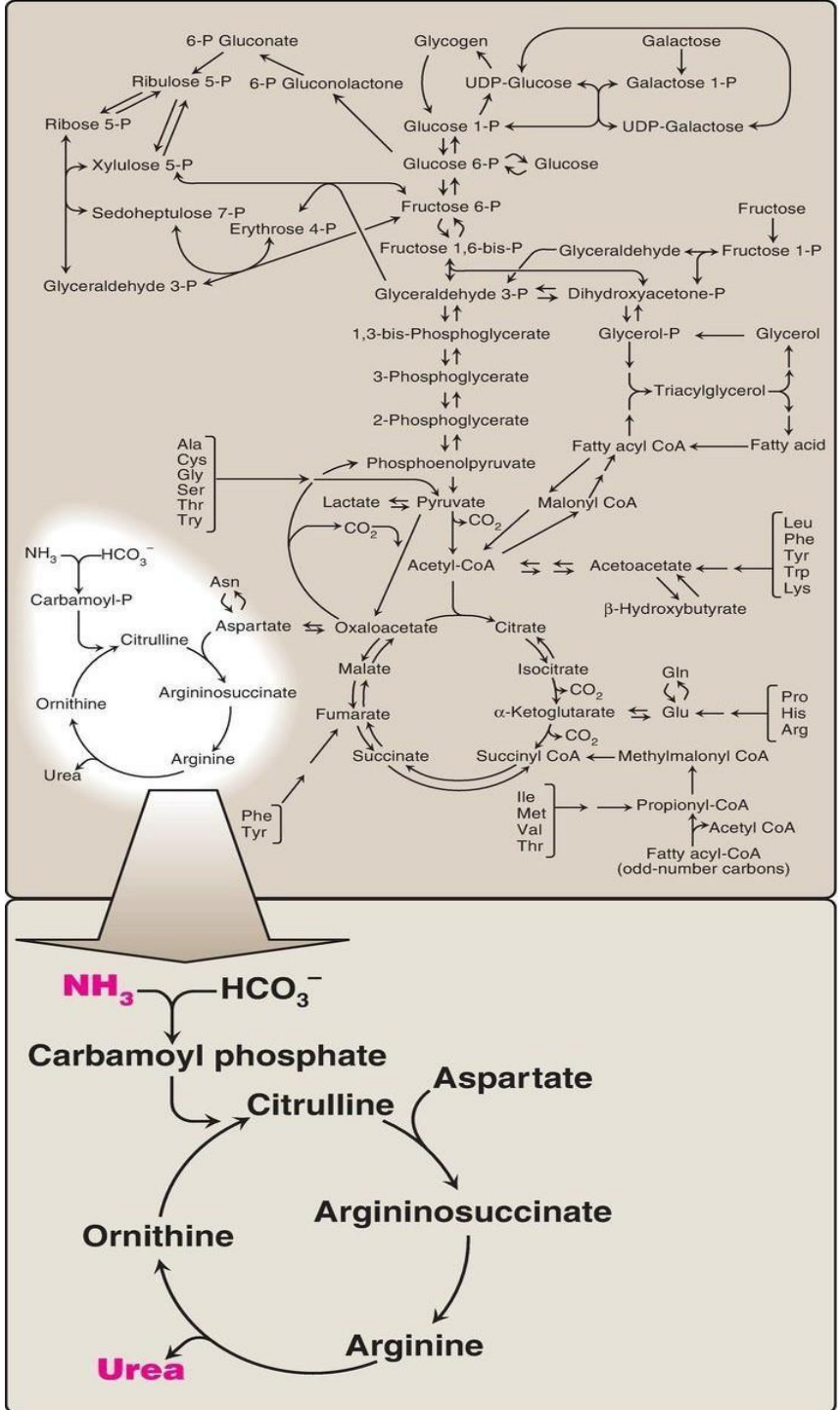
Protein Metabolism: From Plate to Portal Vein

- Learning Objectives:
  - Trace protein digestion from plate to absorption
  - Explain transport mechanisms for amino acids and peptides
  - Calculate and interpret nitrogen balance
  - Recognize clinical disorders of protein digestion



OVERVIEW

- Unlike fats and carbohydrates
- The first phase
  - *A portion excreted in the urine, but most as urea*
- The second phase
  - *Metabolized to carbon dioxide (CO_2) and water (H_2O), glucose, fatty acids, or ketone bodies by the central pathways of metabolism*



Overview

- Nitrogen exists in the body in a large amount in what are called nitrogen-containing compounds, mainly proteins.
- Nitrogen metabolism is linked to proteins because the building blocks of proteins are amino acids, which are the source of nitrogen in our body.

- **Why nitrogen is important:**
  1) It exists in the nitrogenous bases of nucleic acids.
  2) It exists in ATP, which is the source of energy in the body.
  3) It is needed for protein synthesis, because proteins are made of amino acids, which contain nitrogen.
  4) It is important in heme synthesis. Heme is composed of iron plus a porphyrin ring, and iron binds specifically to the nitrogen atoms of this ring. We make porphyrins of heme in our body, for example hemoglobin in red blood cells, which are reformed approximately every three months.

- **How proteins are different from fats and carbohydrates:**
  1) Proteins are not stored in the body. If you utilize the incoming amino acids and proteins, you can use them; otherwise, you have to discard them or convert them to another form. Increased amounts of proteins without utilization are harmful.

  2) Amino acids can be converted to forms that give energy, and in the end this may increase adiposity and make you fat.

Overview

How we deal with proteins:

Proteins enter our body from different sources such as meat, eggs, and cereals, and then they are broken down into amino acids.

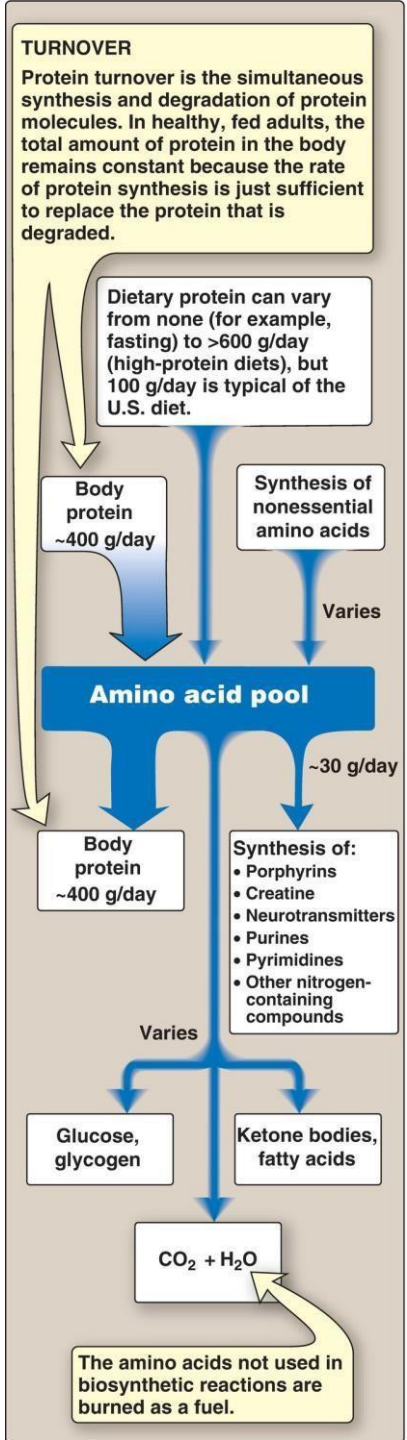
To deal with amino acids, there are two stages:

- The first stage is the same for all amino acids regardless of their nature, and it involves dealing with the amino group, which includes nitrogen. The amino group must be removed from the amino acid, so we end up with a carbon skeleton.
- In the second stage, we deal with the carbon skeleton, and this depends on the type of amino acid. The body deals with them differently, and accordingly there will be different metabolites produced from the carbon skeleton, which leads to the classification of amino acids.

OVERALL NITROGEN METABOLISM

Amino acid pool

- What is it?
- Sources? 3
- Depletion? 3
- ~90–100 g (~10-12 kg in 70 kg man)
- In healthy, well-fed individuals (steady state), and the individual is said to be in nitrogen balance



Overview

Amino acid pool means the total amount (concentration) of free amino acids in the body.
These free amino acids exist in the blood and also in the extracellular fluid.
>>The total amount of free amino acids is about 90–100 grams in a normal adult man who weighs around 70 kg and has 10–12 kg of muscle mass.
>>The amino acid pool varies all the time, because amino acids are continuously being taken out of the pool and supplied back into it.

Sources That Supply the Amino Acid Pool

There are three main sources:

1. Dietary proteins

Proteins are broken down into amino acids, absorbed through the intestine, and enter the circulation.
Some amino acids go into the cells, while others remain in the blood.

2. Degradation of body proteins

Proteins throughout the body are continuously broken down, whether in tissues, extracellular fluid, or blood.

3. Synthesis of amino acids

This applies to non-essential amino acids, which can be synthesized by the body.

Processes That Remove Amino Acids from the Pool

There are three main processes:

1) Protein synthesis
2) Metabolism of amino acids
3) Conversion of amino acids into other molecules

OVERALL NITROGEN METABOLISM
Protein turnover

- Vary from a protein to another
- Rate: the total amount of protein in the body remains constant (hydrolysis and resynthesis of 300–400 g of body protein daily)
- The rate of protein turnover varies:
  - *Shortlived proteins (regulatory proteins and misfolded proteins, minutes or hours)*
  - *Long-lived proteins (days to weeks, majority of proteins in the cell)*
  - *Structural proteins (months or years)*
- Clinical correlate: CRP half-life 19 hours - rapid marker of inflammation

Overview

Nitrogen Balance

In a healthy adult, the body is normally in nitrogen balance, meaning that the amount of nitrogen entering the body equals the amount leaving it, with only minimal differences.
This state is called zero nitrogen balance.

You may be in a positive nitrogen balance, meaning you are gaining more nitrogen than you are losing. This occurs in situations such as high protein intake or pregnancy. During pregnancy, the woman must be in positive nitrogen balance because the fetus also requires proteins taken from the mother.

You may also be in a negative nitrogen balance, meaning you are losing more nitrogen than you are gaining.
This happens in conditions such as starvation, where muscle wasting occurs due to protein degradation.
It also occurs in severe illness, trauma, or burns, where large amounts of proteins are lost.

Conclusion

Positive or negative nitrogen balance can occur in physiological or pathological states.

Why Do We Degrade Proteins?

Proteins are degraded because each protein has its own half-life. The half-life differs between proteins and reflects the function required from each one. Some proteins are needed only for a short period, while others are required for long periods.

Accordingly, proteins can be classified based on their degradation time into:

- Short-lived proteins: Usually involved in controlling metabolic pathways.
- Medium-lived proteins
- Long-lived proteins: Regulate organ processes.
- Structural proteins: Have very long half-lives (years).

➢ Proteins must be degraded so that the body can shut off the metabolic pathways they are controlling. Therefore, according to their function, proteins must have different degradation times.

Clinical Use of Protein Half-Life

- The concept of protein half-life and degradation is used clinically.

Albumin and Prealbumin

Albumin and prealbumin are used to reflect liver function. About 25% of liver capacity goes toward albumin synthesis, so a decrease in albumin concentration suggests impaired liver function. However, albumin has a relatively long half-life (about 20 days), so changes take time to become detectable.

Therefore, prealbumin is better for assessment because it has a shorter half-life (about 2 days), allowing faster evaluation of liver function. The problem with prealbumin is that its concentration in plasma is very low.

- This demonstrates that protein half-life and degradation are important clinical concepts.

C-Reactive Protein (CRP)

C-reactive protein (CRP) is a plasma protein and a universal marker of inflammation. It is considered an acute-phase protein and increases in conditions such as acute inflammation, cancer, trauma, and chronic inflammation.

Although CRP is not specific, it indicates that a pathological process is present, and the patient may need hospital admission. The half-life of CRP is about 19 hours.

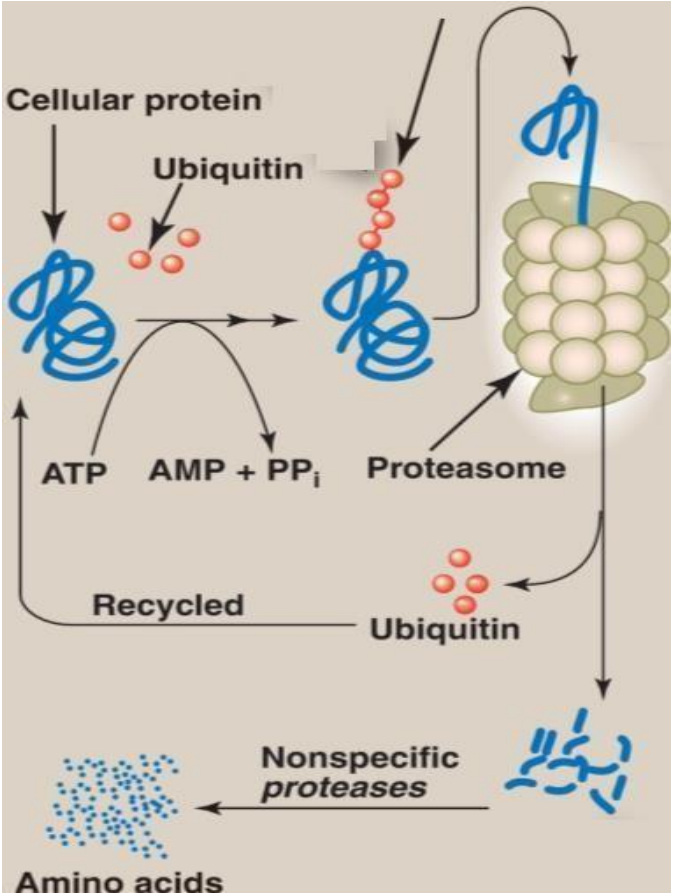
✓ Remember: CRP reaches its peak approximately 2 days after the onset of inflammation.

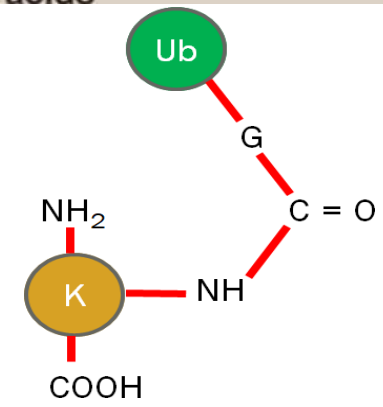
OVERALL NITROGEN METABOLISM
Protein turnover

- Protein degradation:
  - *ATP-dependent ubiquitin (Ub)–proteasome system of the cytosol (selective, damaged or short-lived proteins)*
  - *ATP-independent degradative enzyme system of the lysosomes (acid hydrolases, nonselective, autophagy and heterophagy)*

Ubiquitin–proteasome system

- A small, globular, non-enzymic, highly conserved
- Covalent attachment, ATP (hydrolytic enzymes), enzyme catalyzed (E1 activates, E2 conjugates, E3 ligates)
- There are many more *E3* proteins (*E1* or *E2)? Why?*
- A polyubiquitin chain (4 or more) is recognized
- Proteasome: large, macromolecular, barrel-shaped, proteolytic complex (unfolds, deubiquitinates, and cuts)
- Cytosolic proteases (amino acid pool)
- Ub is recycled





Degradation signals

- Is protein degradation a random process? Why? How?
- Recognized and bound by an *E3*
- N-end rule, and ranges from minutes to hours.
- Destabilizing (Arg, acetylated alanine), Stabilizing (Ser)
- Proteins rich PEST sequences are rapidly ubiquitinated and degraded

Protein Degradation

- We focus on protein degradation because protein synthesis occurs according to the needs of the body. If the body needs a protein, it synthesizes it; however, protein degradation is a selective process.

- Protein degradation differs according to the location of the protein, whether it is extracellular or intracellular.

1. Degradation of Extracellular Proteins

Proteins outside the cell must first enter the cell by endocytosis in the form of vesicles. This process is called vesicular transport.

These vesicles then fuse with lysosomes, where hydrolytic enzymes inside the lysosome break the proteins down.

This process does not require energy and is ATP-independent.

2. Degradation of Intracellular Proteins

The previous pathway does not apply to proteins inside the cell. Therefore, intracellular proteins are degraded by a specific system called the ubiquitin-proteasome system.

The proteasome is a large machinery in the cytoplasm that looks like an empty barrel. It contains hydrolytic enzymes that can break down proteins. When a protein enters the proteasome, it is degraded.

How Does a Protein Enter the Proteasome?

Proteins that will enter the proteasome must be tagged for degradation.

This tag is called ubiquitin.

Ubiquitin is a small, non-enzymatic globular protein that is attached to proteins destined for degradation.

Attachment of Ubiquitin

- The attachment of ubiquitin requires the action of three enzyme systems:

  1. Activating enzymes (E1)
  2. Conjugating enzymes (E2)
  3. Ligating enzymes (E3)

- The activating enzyme activates ubiquitin, conjugating enzymes link ubiquitin molecules together, and ligating enzymes attach ubiquitin to the target protein.

- Variation exists mainly in the ligating enzymes, because they must recognize a very large number of different proteins. Therefore, there are more than 600 ligating enzymes, while there are only two activating enzymes and about 30–40 conjugating enzymes.

- At least four ubiquitin molecules must be attached to a protein for it to be recognized by the proteasome.

Degradation Inside the Proteasome

- The ubiquitinated protein enters the proteasome, where it is unfolded, and then hydrolytic enzymes cut it into small peptides.
- These peptides exit the proteasome and are further broken down by non-specific proteases in the cytosol into free amino acids.
- Ubiquitin is then recycled and reused.

Specificity of Ubiquitin Attachment

- Ubiquitin contains a glycine residue, which requires lysine residues on the target protein for attachment. Therefore, the system recognizes the presence of lysine residues on the protein of interest.
- Ubiquitin is attached to specific proteins because those proteins contain signals within their primary structure that indicate they should be degraded.
- The primary structure of a protein determines its function, shape, final form, and the time at which it will be degraded.

Protein Degradation and Protein Digestion

The primary structure of a protein (its amino acid sequence) determines how and when the protein will be degraded.

Protein Degradation: The N-end Rule

- Each protein has an N-terminus and a C-terminus.According to the N-end rule, the amino acid present at the N-terminal end acts as a signal that determines whether the protein is stable or unstable.
- Some N-terminal residues signal that the protein should be rapidly degraded.
- Other residues indicate that the protein is stable and should persist for a longer time.
- If a protein is stable, the enzymes involved in degradation cannot easily attach to it.

Examples of N-terminal Residues

- Arginine at the N-terminus is a destabilizing residue and signals rapid degradation.
- Alanine, when acetylated (acetylated alanine with an acetyl group), signals rapid degradation.
- Serine at the N-terminus is a stabilizing residue, meaning the protein should not be degraded quickly.
- PEST sequences (rich in Proline, Glutamic acid, Serine, and Threonine) are destabilizing sequences that signal rapid protein degradation.

✓ These examples demonstrate that the primary structure of a protein plays a key role in controlling the protein degradation process.

DIETARY PROTEIN DIGESTION

Where we start?

Gastric Phase - More Than Acid

Parietal cells: H^+/K^+ ATPase (proton pump)

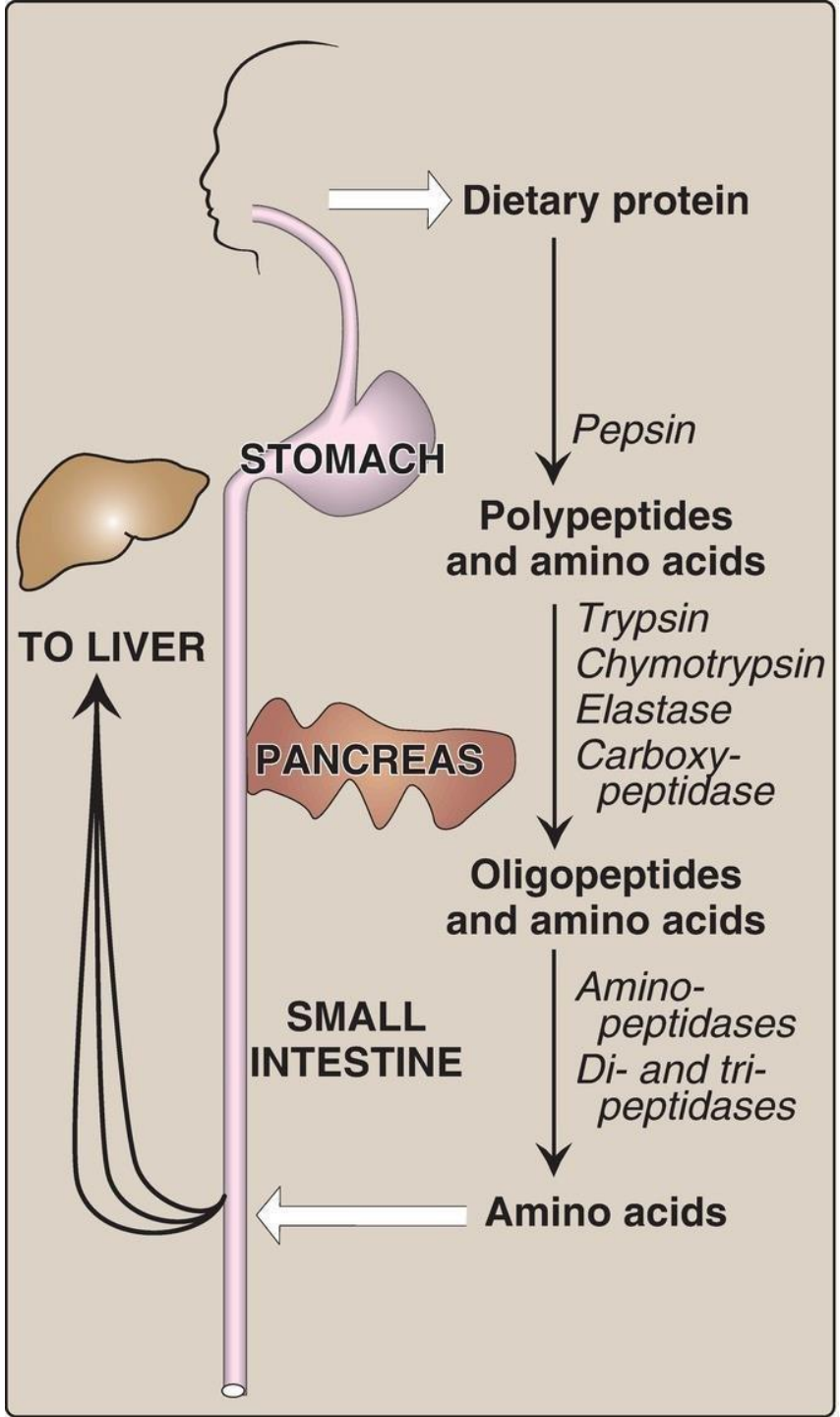
Acid functions:

- Denaturation
- Activates pepsinogen → pepsin
- Bactericidal

Pepsin characteristics:

- Optimal pH 1.5-2.5, inactive above pH 5.0
- Endopeptidase (preference for AAA)

Gastric lipase and amylase also present



Entero-endocrine Regulation

G cells: Gastrin → stimulates acid and pepsinogen

D cells: Somatostatin → inhibits gastrin, acid

I cells: CCK → gallbladder contraction, pancreatic secretion

S cells: Secretin → bicarbonate secretion, inhibits acid

Other Sources of Amino Acids: Dietary Proteins

- Amino acids are not obtained only from protein degradation inside the cell. They are also obtained from dietary proteins, which must first be digested.

Oral and Gastric Phases of Protein Digestion

- Protein digestion begins with mechanical digestion in the mouth through chewing, which physically breaks food into smaller pieces.

- Chemical digestion of proteins begins in the stomach.

In the Stomach

- The acidic pH causes protein denaturation by altering the protein structure.

- Gastric acid also kills microorganisms such as bacteria, fungi, and viruses, acting as a protective mechanism.

Pepsin and Pepsinogen

- Pepsinogen is secreted as an inactive zymogen.

- Due to the acidic environment and its high concentration, a small portion of pepsinogen is converted into pepsin.

Once pepsin is formed:

- Pepsin activates additional pepsinogen molecules (autocatalysis).

- Pepsin is a protease that recognizes specific sequences and cleaves peptide bonds.

- Pepsin primarily acts on peptide bonds near aromatic amino acids, functioning as an endopeptidase.

Intestinal Phase of Protein Digestion

After the stomach, food moves into the small intestine, specifically the duodenum.

The duodenum has important anatomical connections:

- **It receives secretions from the pancreas and the liver through the pancreatic duct and the bile duct.**

- **These ducts join to form the common bile duct, which opens into the duodenum.**

✓ **These secretions support the digestion of nutrients, including proteins.**

Hormonal Regulation in the Duodenum

The duodenum contains specialized endocrine cells that regulate digestion:

- **G cells secrete gastrin, which stimulates the stomach to secrete hydrochloric acid and pepsinogen when food enters the gastrointestinal tract.**
  (a positive feedback mechanism).

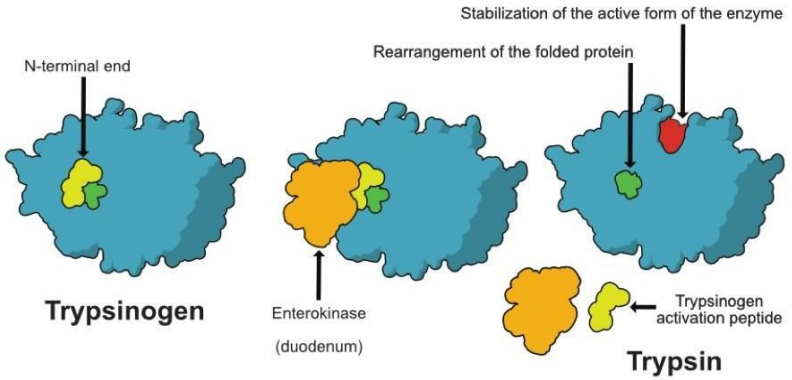
- **D cells secrete somatostatin, which inhibits gastric secretions when they are no longer needed.**

- **I cells secrete cholecystokinin (CCK), which stimulates the pancreas to release digestive enzymes.**

- **S cells stimulate the pancreas to secrete bicarbonate, which neutralizes the acidic chyme coming from the stomach into the duodenum.**

Pancreatic Protease Cascade

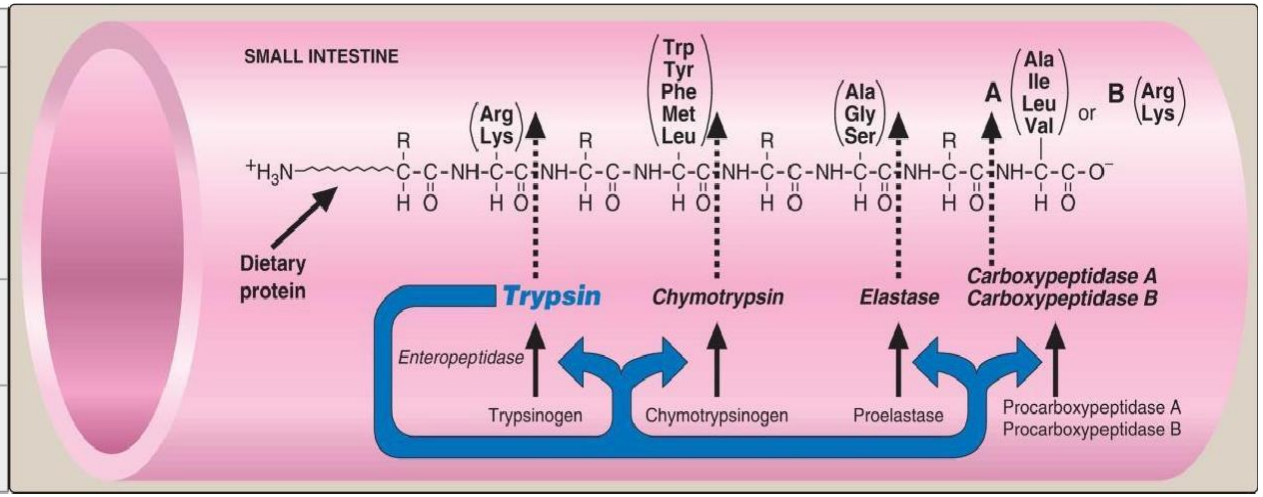
- Zymogen activation sequence:
- Enteropeptidase (brush border) cleaves trypsinogen → trypsin
- Trypsin activates:
  - More trypsinogen (autocatalysis)
  - Chymotrypsinogen → chymotrypsin
  - Proelastase → elastase
  - Procarboxypeptidase A/B → carboxypeptidase A/B
- Trypsin inhibitor (pancreatic) prevents premature activation
- Clinical: Enteropeptidase deficiency causes protein malabsorption, diarrhea



Enzyme Specificity

- Trypsin: after Lys, Arg (basic)
- Chymotrypsin: after Phe, Tyr, Trp, Leu (aromatic/bulky)
- Elastase: after Ala, Gly, Ser (small neutral)
- Carboxypeptidase A: removes hydrophobic C-terminal
- Carboxypeptidase B: removes basic C-terminal

Enzyme	Specificity
Trypsin	C-terminal to R, K, but not if next to P
Chymotrypsin	C-terminal to F, Y, W but not if next to P
Elastase	C-terminal to A, G, S, V, but not if next to P
Pepsin	N-terminal to L, F, W, Y, but not when next to P



Brush Border Enzymes - The Final Cut

- Aminopeptidase N: removes N-terminal aa, zinc metalloenzyme
- Aminopeptidase A: prefers acidic N-terminal
- Dipeptidyl peptidase IV: cleaves after Pro (collagen)
- Enteropeptidase: $(Asp)_4$-Lys (trypsinogen) – research tags
- Clinical: Brush border enzyme deficiencies cause intolerance to specific proteins

Pancreatic Proteases and Their Activation

- The enzymes secreted by the pancreas into the intestine include trypsinogen, chymotrypsinogen, proelastase, and procarboxypeptidases (types A and B).

- All of these enzymes are secreted as zymogens, which are inactive precursors that require activation in the intestine to prevent autodigestion of the pancreas.

Activation of Pancreatic Zymogens

- Activation occurs on the brush border of intestinal mucosal cells by an enzyme called enteropeptidase.

- Enteropeptidase specifically recognizes a sequence of four aspartic acid residues followed by lysine (Asp-Asp-Asp-Asp-Lys) in trypsinogen and cleaves it to form active trypsin.

- Once trypsin is activated, it acts as the key enzyme that activates the other pancreatic zymogens:
  - Trypsinogen → Trypsin
  - Chymotrypsinogen → Chymotrypsin
  - Proelastase → Elastase
  - Procarboxypeptidases → Active carboxypeptidases

Specificity of Proteolytic Enzymes

- Each enzyme cleaves peptide bonds at specific amino acids:
  - Trypsin cleaves peptide bonds after arginine and lysine.
  - Chymotrypsin cleaves peptide bonds after aromatic amino acids.
  - Elastase cleaves peptide bonds after small neutral amino acids, such as alanine, glycine and serine .

- Pepsin digests approximately 40% of dietary proteins.

- Carboxypeptidases are exopeptidases that remove amino acids one at a time from the C-terminal end of peptides.

- The resulting smaller peptides are further digested by aminopeptidases located on the brush border, which remove amino acids from the N-terminal end.

- Aminopeptidase A preferentially removes negatively charged amino acids, such as aspartate and glutamate.

- Dipeptidyl peptidases preferentially digest proline-containing peptides, which are abundant in collagen, the most abundant protein in the body.

Clinical Correlation

- Diseases that affect the intestine, such as celiac disease, can damage the brush border and intestinal epithelial cells, leading to reduced secretion of enzymes such as enteropeptidase.

- This results in impaired activation of pancreatic enzymes, decreased protein hydrolysis, and reduced nutrient absorption, ultimately causing malabsorption and nutritional deficiencies.

رسالة من الفريق العلمي:

عَنِ النَّبيِّ صَلَّى اللَّهُ عليه وسلَّمَ فِيما رَوَى عَنِ اللهِ تَبَارَكَ وَتَعَالَى، أَنَّهُ قالَ: **يا عِبَادِي**، إِنِّي حَرَّمْتُ الظُّلْمَ علَى نَفْسِي، وَجَعَلْتُهُ بيْنَكُمْ مُحَرَّمًا، فلا تَظَالَمُوا، **يا عِبَادِي**، كُلُّكُمْ ضَالٌّ إلَّا مَن هَدَيْتُهُ، فَاسْتَهْدُونِي أَهْدِكُمْ، **يا عِبَادِي**، كُلُّكُمْ جَائِعٌ إلَّا مَن أَطْعَمْتُهُ، فَاسْتَطْعِمُونِي أُطْعِمْكُمْ، **يا عِبَادِي**، كُلُّكُمْ عَارٍ إلَّا مَن كَسَوْتُهُ، فَاسْتَكْسُونِي أَكْسُكُمْ، **يا عِبَادِي**، إنَّكُمْ تُخْطِئُونَ باللَّيْلِ وَالنَّهَارِ، وَأَنَا أَغْفِرُ الذُّنُوبَ جَمِيعًا، فَاسْتَغْفِرُونِي أَغْفِرْ لَكُمْ، **يا عِبَادِي**، إنَّكُمْ لَنْ تَبْلُغُوا ضَرِّي فَتَضُرُّونِي، وَلَنْ تَبْلُغُوا نَفْعِي فَتَنْفَعُونِي، **يا عِبَادِي**، لو أنَّ أَوَّلَكُمْ وَآخِرَكُمْ وإِنْسَكُمْ وَجِنَّكُمْ، كَانُوا علَى أَتْقَى قَلْبِ رَجُلٍ وَاحِدٍ مِنكُمْ؛ ما زَادَ ذلكَ في مُلْكِي شيئًا، **يا عِبَادِي**، لوْ أنَّ أَوَّلَكُمْ وَآخِرَكُمْ وإِنْسَكُمْ وَجِنَّكُمْ، كَانُوا علَى أَفْجَرِ قَلْبِ رَجُلٍ وَاحِدٍ؛ ما نَقَصَ ذلكَ مِن مُلْكِي شيئًا، **يا عِبَادِي**، لو أنَّ أَوَّلَكُمْ وَآخِرَكُمْ وإِنْسَكُمْ وَجِنَّكُمْ، قَامُوا في صَعِيدٍ وَاحِدٍ فَسَأَلُونِي، فأعْطَيْتُ كُلَّ إنْسَانٍ مَسْأَلَتَهُ؛ ما نَقَصَ ذلكَ ممَّا عِندِي إلَّا كما يَنْقُصُ المِخْيَطُ إذَا أُدْخِلَ البَحْرَ، **يا عِبَادِي**، إنَّما هي أَعْمَالُكُمْ أُحْصِيهَا لَكُمْ، ثُمَّ أُوَفِّيكُمْ إيَّاهَا، فمَن وَجَدَ خَيْرًا فَلْيَحْمَدِ اللَّهَ، وَمَن وَجَدَ غيرَ ذلكَ فلا يَلُومَنَّ إلَّا نَفْسَهُ.

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Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
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V1 → V2			