Proteins/Amino Acids
Protein metabolism

By

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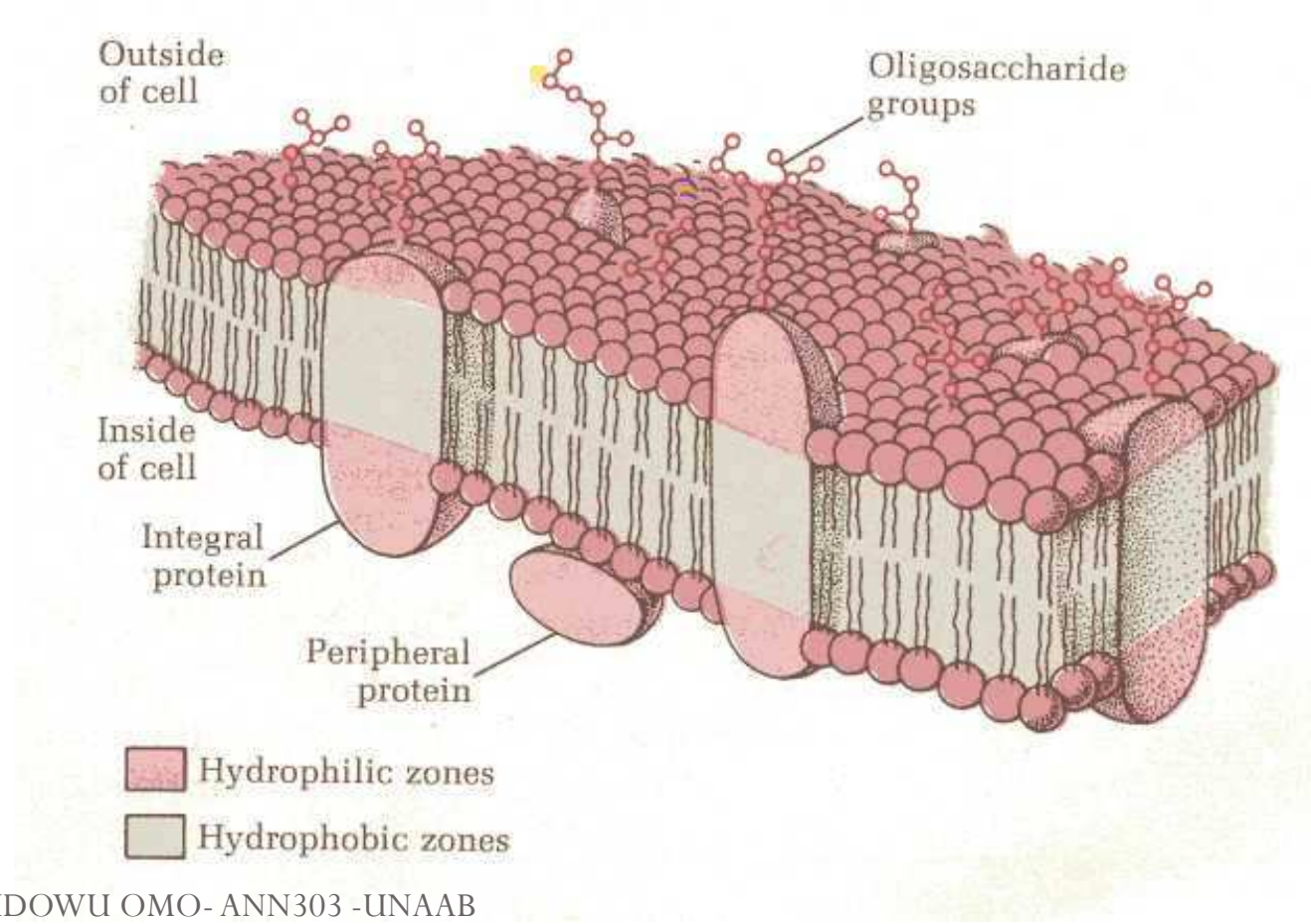
Preliminary Concepts

- Protein is the principal constituent of organs and soft structures of the animal body
- a continuous supply is needed from feed sources throughout life for growth/repair
- food protein → body protein
- food protein: plant or animal
- unique proteins found in each animal
- no two are alike in physiological behavior

Roles of Protein

1. bulk composition of the body (structural aspects of the cell)
2. oxidative metabolism (used as energy source in energy-deficient diets)
3. enzymes (globular proteins that regulate and influence metabolism)
4. plasma proteins (circulating, mobile proteins such as immune bodies)
5. hormones (regulatory role)

Proteins within Cell Wall



Special Functions

- peptides (formation of proteins)
- purines/pyrimidines (control of protein synthesis)
- histamines (active compounds, allergies)
- conjugated proteins (assist in the excretion of other compounds)
- pigments (e.g., melanin, derived from amino acids)

Elementary Composition of Proteins

- Chemical composition: primarily carbon, hydrogen, oxygen
- additional difference: contain a fairly constant amount of nitrogen found in amino groups (17%)
- many also contain sulfur, phosphorus and iron
- structure is typically complex, having high molecular weight

Protein Classification

- **simple proteins**: essentially pure proteins, when **hydrolyzed**, produce individual amino acids (e.g., egg albumin)
- **conjugated**: protein unit linked to another non-protein unit (e.g., **casein**, the protein component of milk with phosphorus esterified to it via the AA serine (ser)
- **derived**: modified proteins such as peptides, modified by heat, acidification,etc.

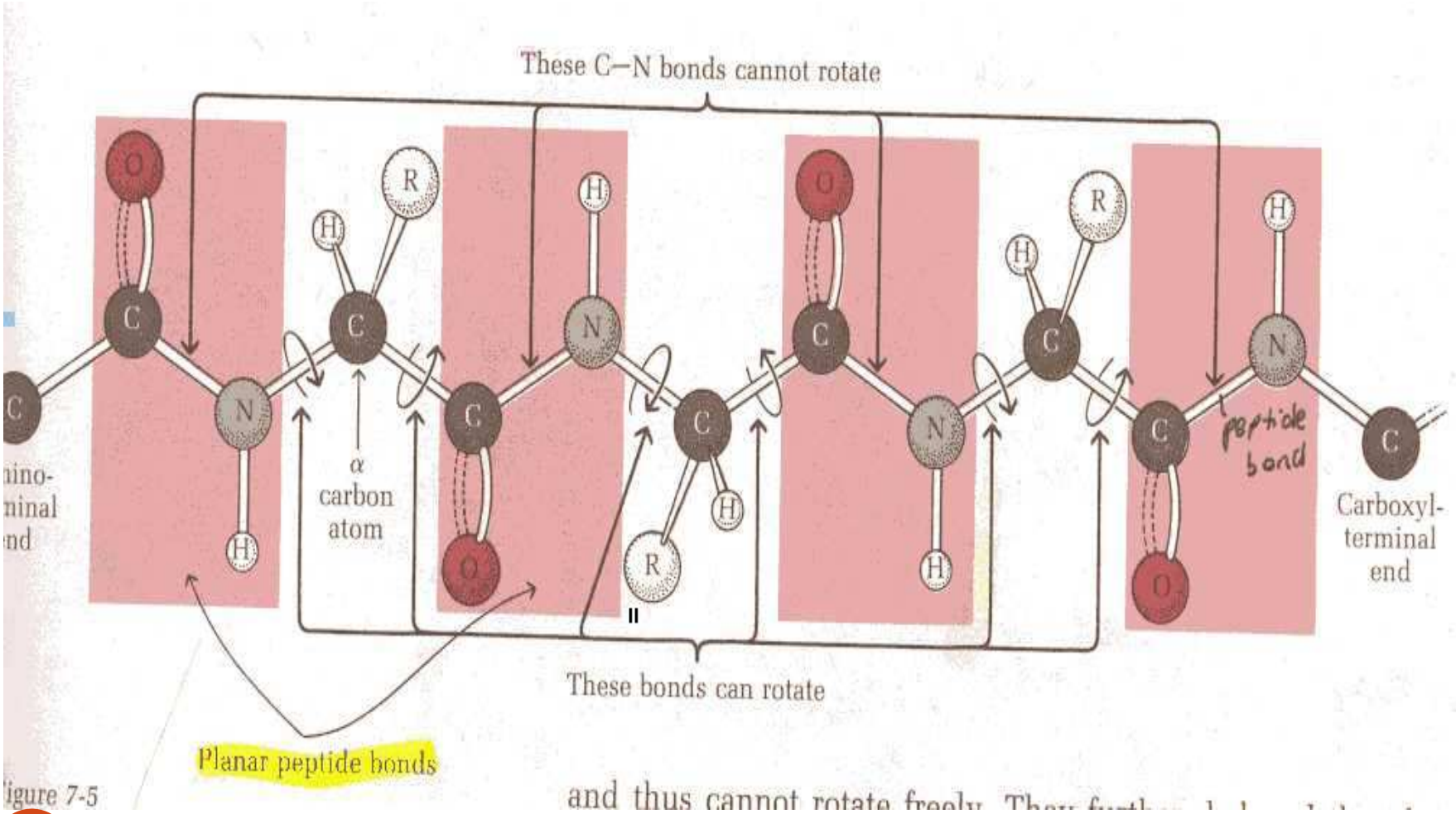
Conjugated Proteins

- **nucleoproteins: protein + nucleic acid (e.g., seed germs)**
- **glycoproteins: protein + COH group (e.g., mucus)**
- **phosphoproteins: protein+ P-containing compound (e.g., casein)**
- **hemoglobins: protein + hematin or similar substance**
- **lecithoproteins: protein + lecithin (e.g., fibrinogen)**

Structure of Protein Molecule

- As mentioned, proteins are sequences of amino acids hooked together by the amino group of one to the carboxyl group of another
- this bond is known as the **peptide linkage**
- AA found in protein are known as **residues**
- protein chains of AA have typically 100-200 residues
- many proteins have more than one chain

The Peptide Linkage



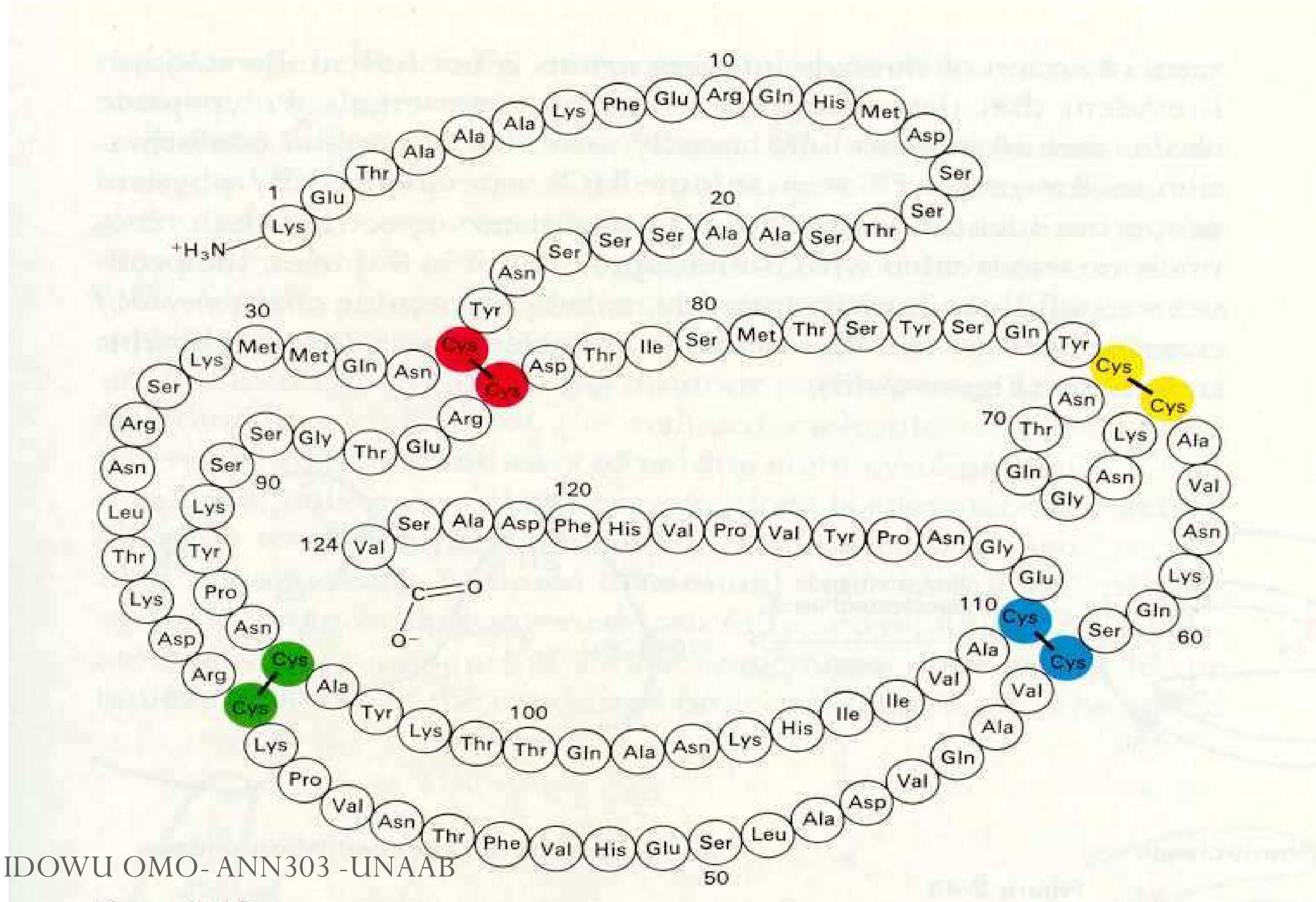
igure 7-5

and thus cannot rotate freely. Ther

Protein Structure

- **primary**: the sequence of AA's forming the protein
- **secondary**: forces generated by the close proximity of one AA residue to another (e.g., α helix design or β pleated sheet)(i.e., certain amino acids can form bonds with others, if close enough, cysteine)
- **tertiary**: bending of one AA chain due to attraction of individual AA's distant from each other
- **quaternary**: packing of chains together

Protein Structure



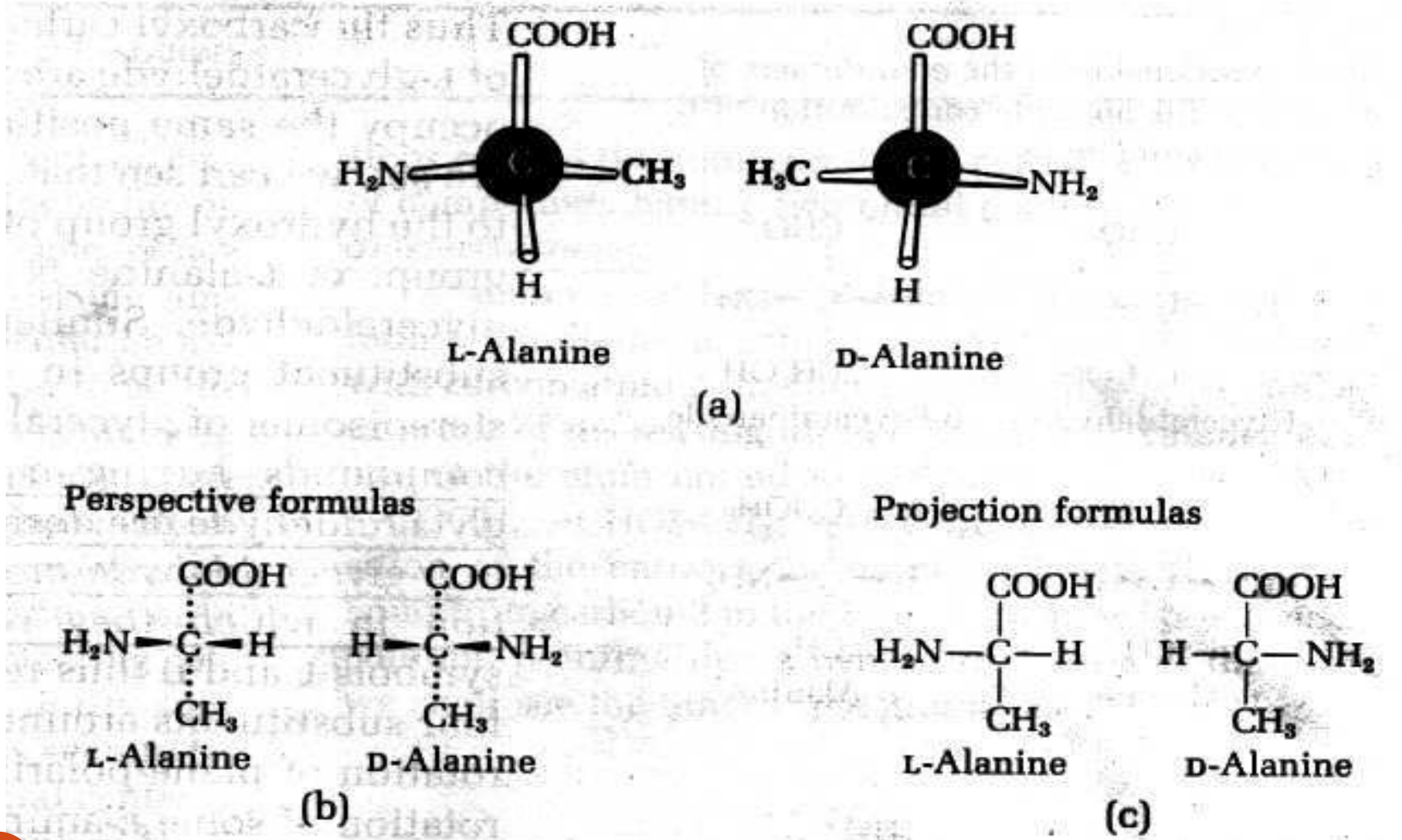
Amino Acids (AA)

- As mentioned, proteins are polymerized residues of amino acids
- the number and proportion of AA vary from protein to protein
- when proteins are denatured, the AA remain
- to study protein, you must study AA
- at least 30 different AA, some essential others non-essential

Characterizing AA

- Most AA are derived from lower or short-chain fatty acids (FA; such as acetic, proprionic or butyric acid)
- naturally-occurring have L-configuration
- synthetic have large proportion of D configs
- soluble in water, amphoteric
- show various types: aliphatic, aromatic, heterocyclic, etc.

“D” vs. “L” Configuration



Aliphatic Amino Acids

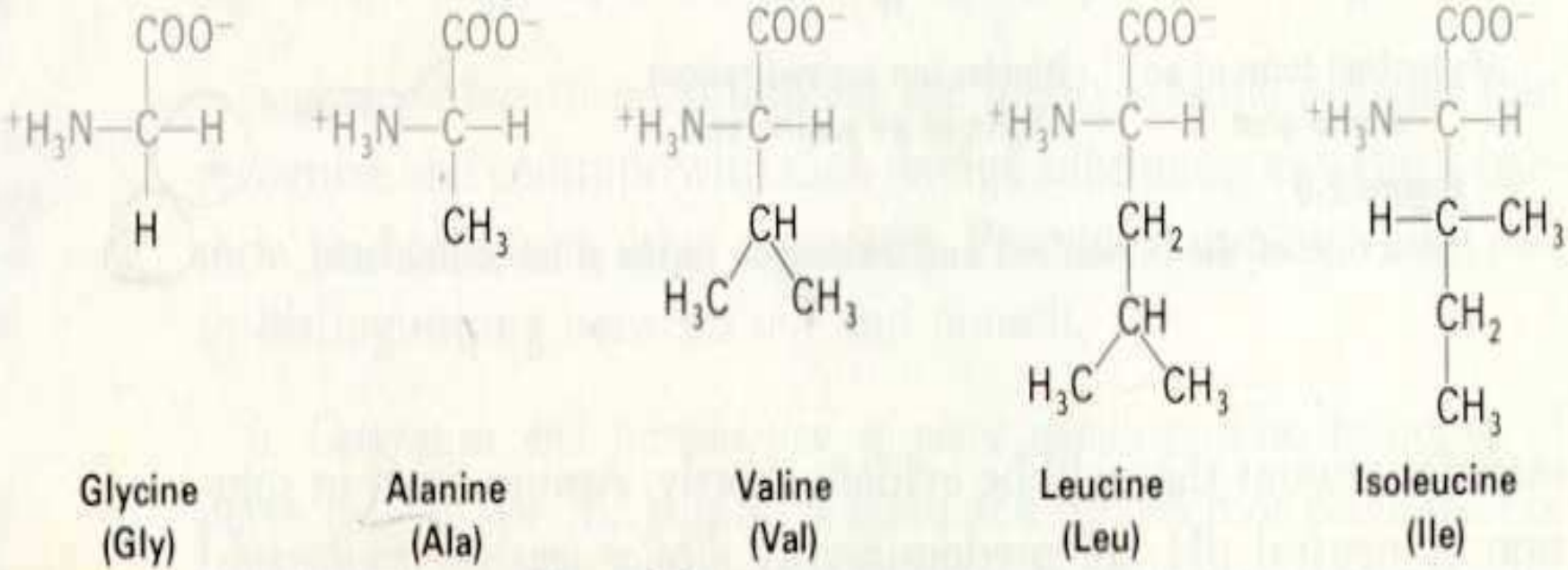


Figure 2-8
Amino acids having aliphatic side chains.

Aromatic Amino Acids

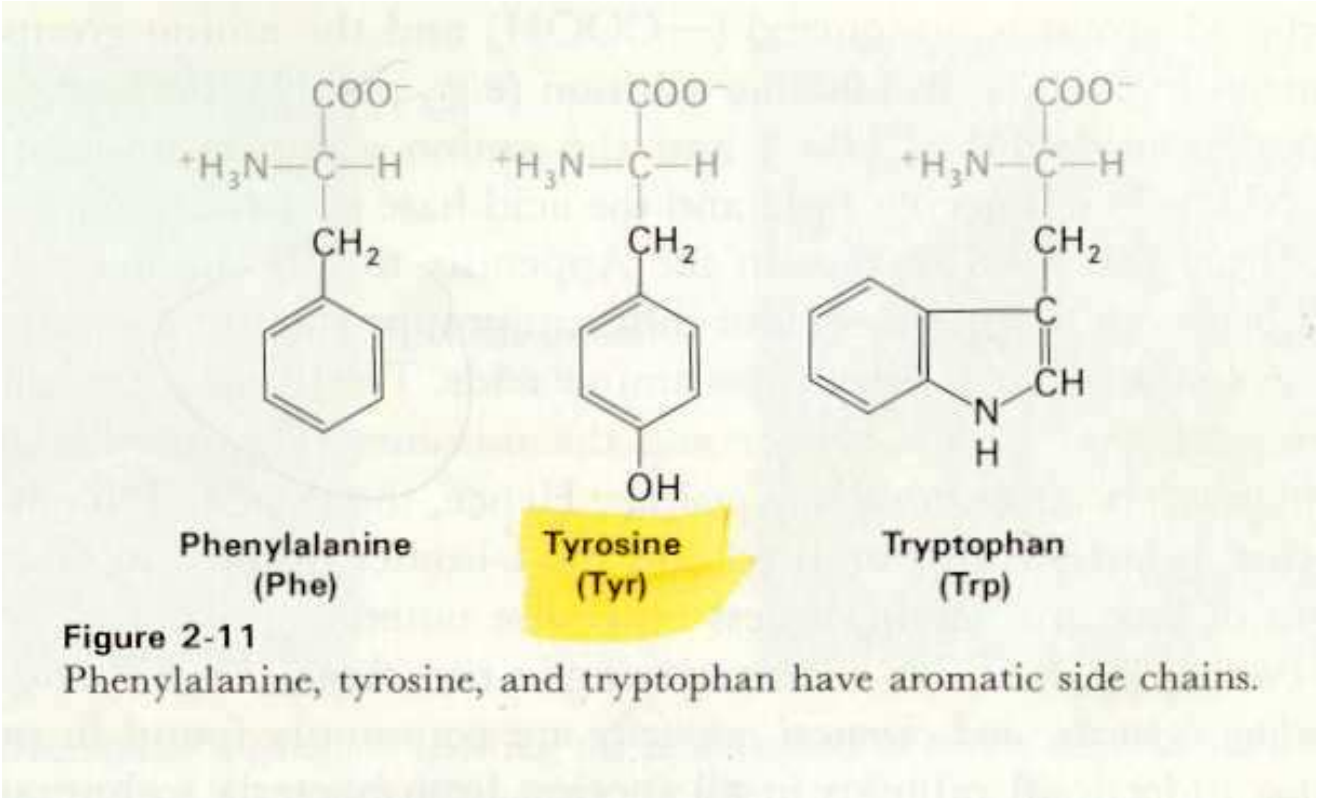


Figure 2-11
Phenylalanine, tyrosine, and tryptophan have aromatic side chains.

Chemical Determination of Protein

- The direct determination of protein in tissue is impractical due to quantity/variation
- nitrogen, however, occurs at fairly constant levels:
- [N] x 6.25 = protein level
- some proteins have well-known nitrogen levels (e.g., milk @ 15.7% N)
- determined by Kjehldal N methodology

Protein/AA Quality

- Amino acids are basically divided into two nutritional categories:
- **essential**: those the animal cannot synthesize in sufficient quantity to support maximum growth, typically dietary in nature
- **nonessential**: synthesized by animal body, typically non-dietary in nature
- determined first by Rose (1930) working on factorial deletion with rats

Essential AA

- **lysine (LYS)**
- **arginine (ARG)**
- **methionine (MET)**
- **histidine (HIS)**
- **isoleucine (ILE)**
- **leucine (LEU)**
- **threonine (THR)**
- **tryptophan (TRY)**
- **phenylalanine (PHE)**
- **valine (VAL)**

Exceptions

ser/gly essential for chicks

pigs don't need ARG, HIS, LEU for maintenance

no big problem for ruminants, why?

All essentials are in "L" form only humans really need HIS

Protein/AA Quantitative Requirements

- A protein requirement is really an EAA requirement (why?)
- unfortunately, not all sources of protein are “balanced”, not all are digestible

EAA Requirements of Chickens and Swine

Amino Acid	Channel Catfish	Tilapia nilotica	Swine
ARG	4.3	4.2	1.2
HIS	1.5	1.7	1.2
ILE	2.6	3.1	3.4
LEU	3.5	3.4	3.7
LYS	5.1	5.1	4.4
MET+ CYS	2.3	3.2	2.3
PHE + TYR	5.0	5.7	4.4
THR	2.0	3.6	2.8
TRY	0.5	1.0	0.8
VAL	3.0	2.8	3.2

Requirements for EAA

- Requirement for one EAA can be partially mitigated by a NEAA
- example: CYS sparing of MET
- CYS replaces about 60% of MET
- often reported as MET-CYS requirement
- example: TYR sparing of PHE (about 50%)

Protein Digestion

What is Digestion?

Digestion is the process by which large, complex nutrient molecules are broken down into simpler molecules capable of being used by the organism for food.

Digestion therefore involves the breakdown of complex food molecules into monomers that can be absorbed and utilized by cells. In most animals, digestion is extracellular, external to the body, occurring in a tubular gut with regions specialized for different digestive functions.

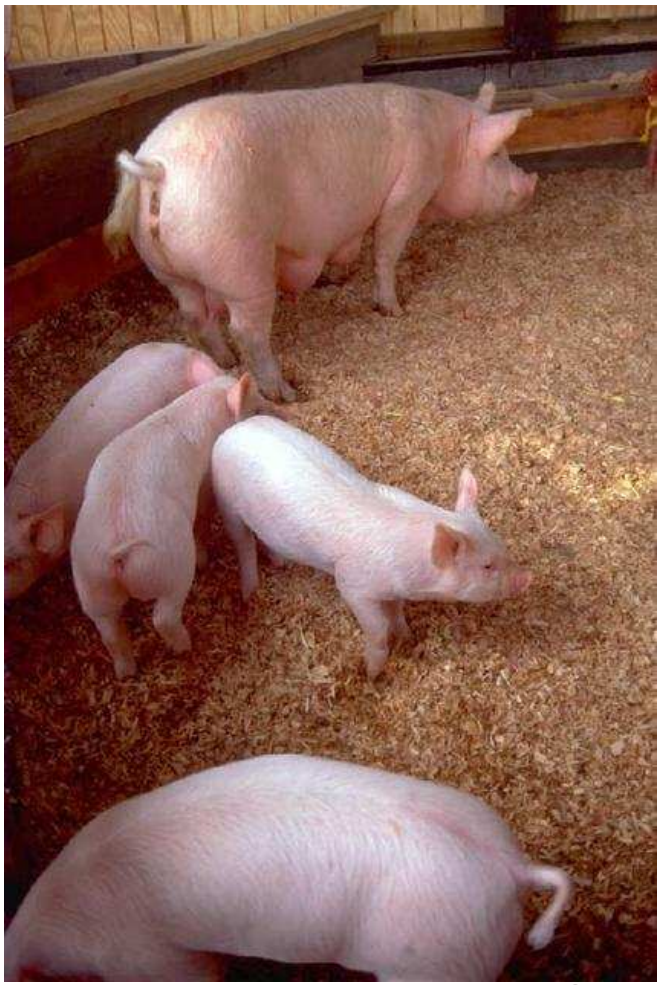
Digestion

- **Digestion:** the preparation of food by the animal for absorption
- involves the following processes:
- 1) mechanical reduction of particle size;
- 2) enzyme solubilization of organics;
- 3) pH solubilization of inorganics;
- 4) emulsification of fats

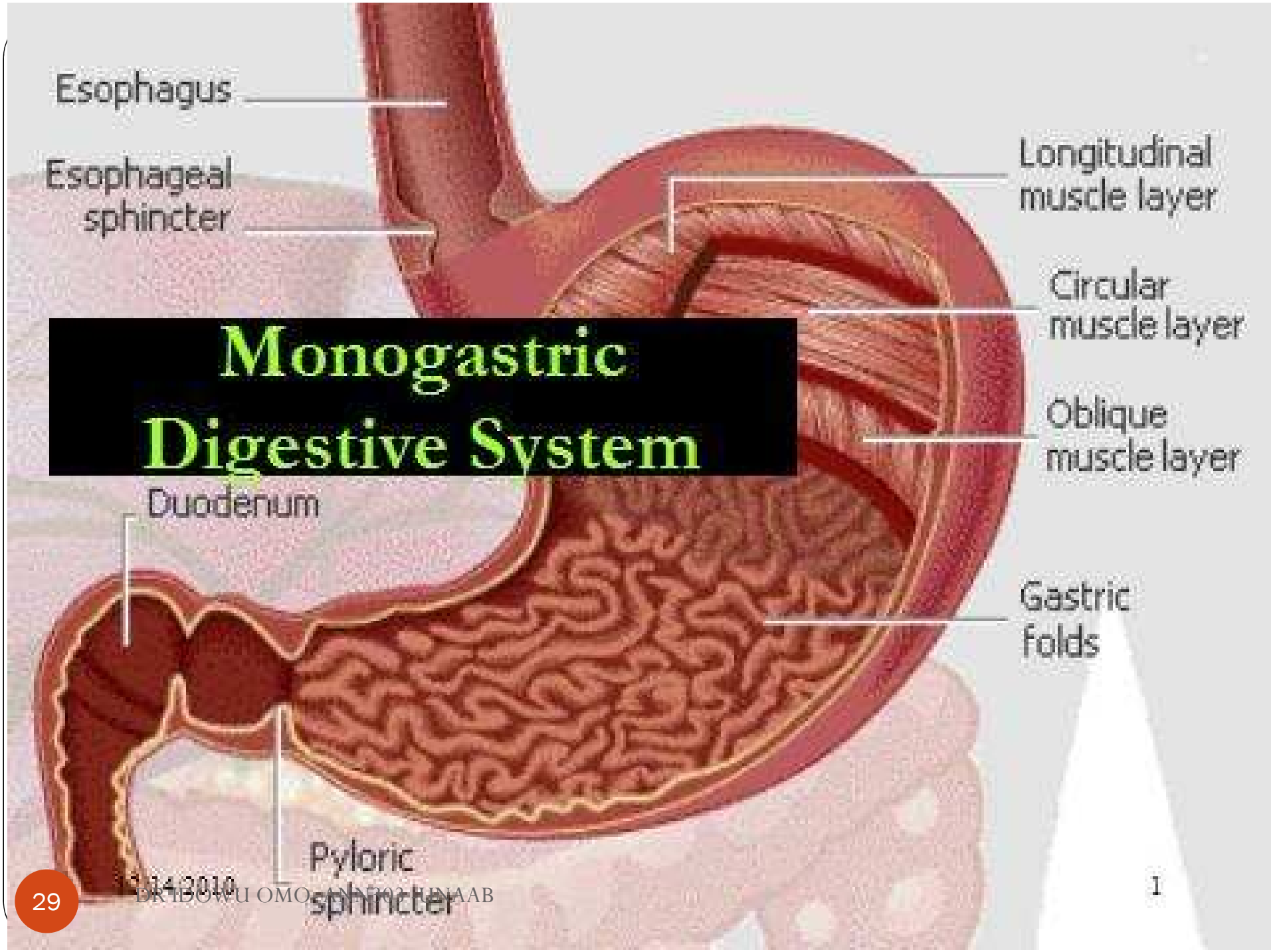
Different Digestive Systems

- The three different types of digestive systems are:
  - Monogastric
  - Modified Monogastric
  - Ruminant

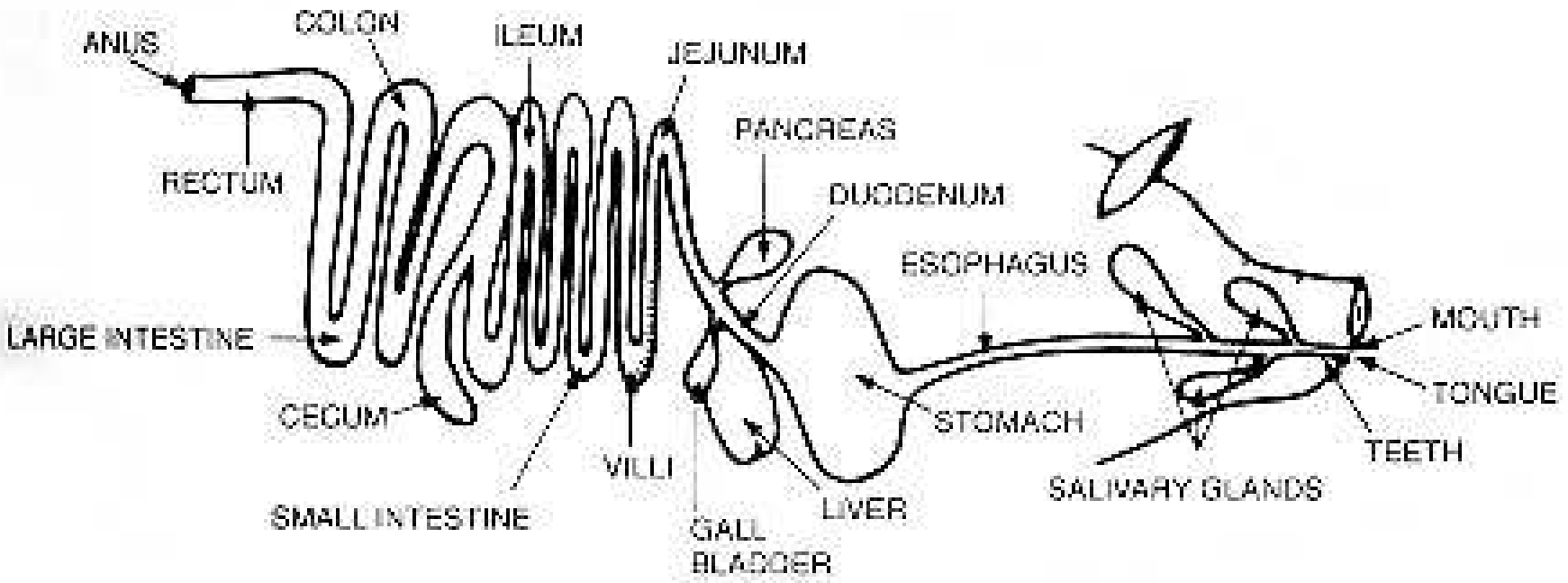
Monogastric

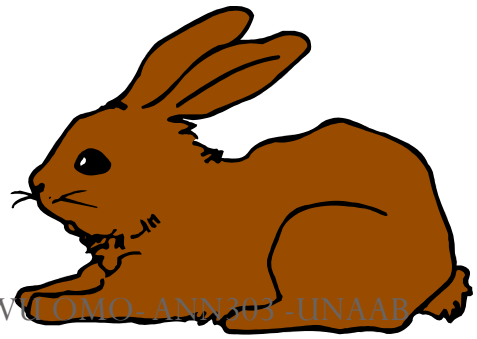


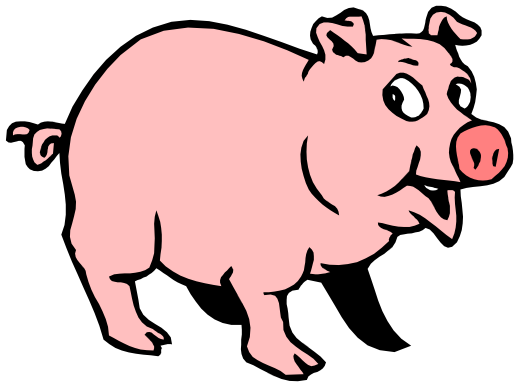
- Carnivores and omnivores have monogastric digestive systems.
- Monogastric systems have a simple stomach structure and only one compartment.
- Some animals with monogastric digestive systems are pigs and rabbits. Humans also have monogastric digestive systems.


Esophagus
Esophageal sphincter
Longitudinal muscle layer
Circular muscle layer
Oblique muscle layer
Monogastric
Digestive System
Duodenum
Gastric folds
Pyloric sphincter

Digestive Tract of the Monogastric Mamma







Parts and functions of the monogastric mammal digestive system continued…

- **Small Intestine**

-enzymatic digestion and absorption

-Functions of the small intestine: digestion of proteins, carbohydrates, and fats; absorption of the end products of digestion

1. **duodenum** - most digestion occurs here
2. **jejunum** - some digestion and some absorption occur
3. **ileum** - mostly absorption

-Bile - made in liver, stored in gall bladder, active in the small intestine, emulsifies fat to aid in digestion

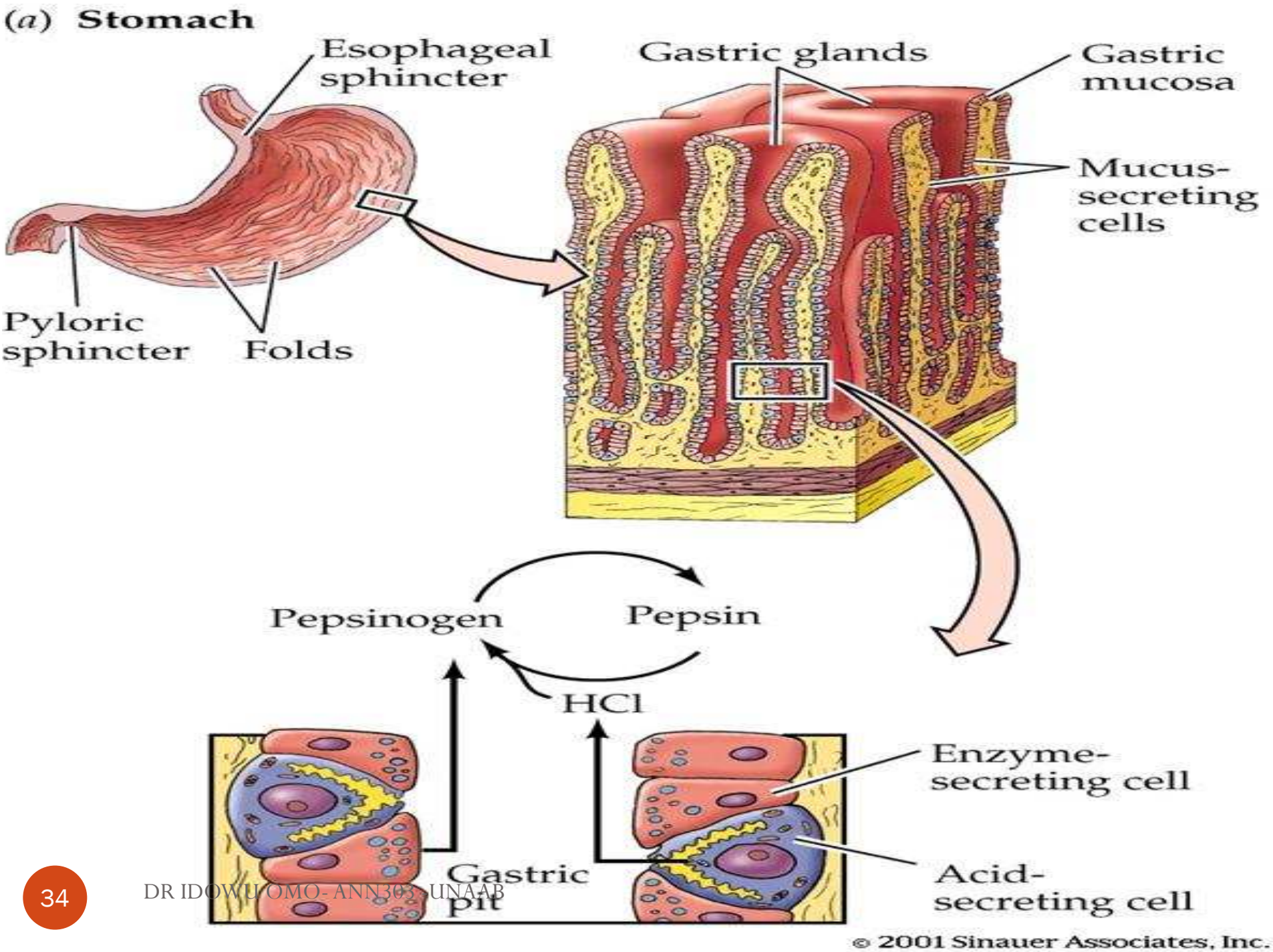
Parts and functions of the monogastric mammal digestive system continued…

- Enzymes in the small intestine

Enzyme	Function	Source
trypsin	digest proteins	secreted from pancreas
chymotrypsin		
carboxypeptides		
pancreatic amylase	digests carbohydrates	secreted from pancreas
lipases	digests lipids	
disaccharides	digests carbohydrates	secreted from small intestine
dipeptidases	digest peptides	

Hydrolytic enzymes break down proteins, carbohydrates, and fats into their monomeric units. To prevent the organism itself from being digested, these are released as inactive zymogens, only activated when secreted into the gut.

Enzymatic digestion begins in the mouth, where amylase is secreted with saliva. Protein digestion begins in the stomach with pepsin and HCl secreted by the stomach mucosa. The mucosa also secretes mucus, to protect gut tissues.


(a) Stomach
Esophageal sphincter
Gastric glands
Gastric mucosa
Mucus-secreting cells
Pyloric sphincter
Folds
Pepsinogen
Pepsin
HCl
Enzyme-secreting cell
Gastric pit
Acid-secreting cell
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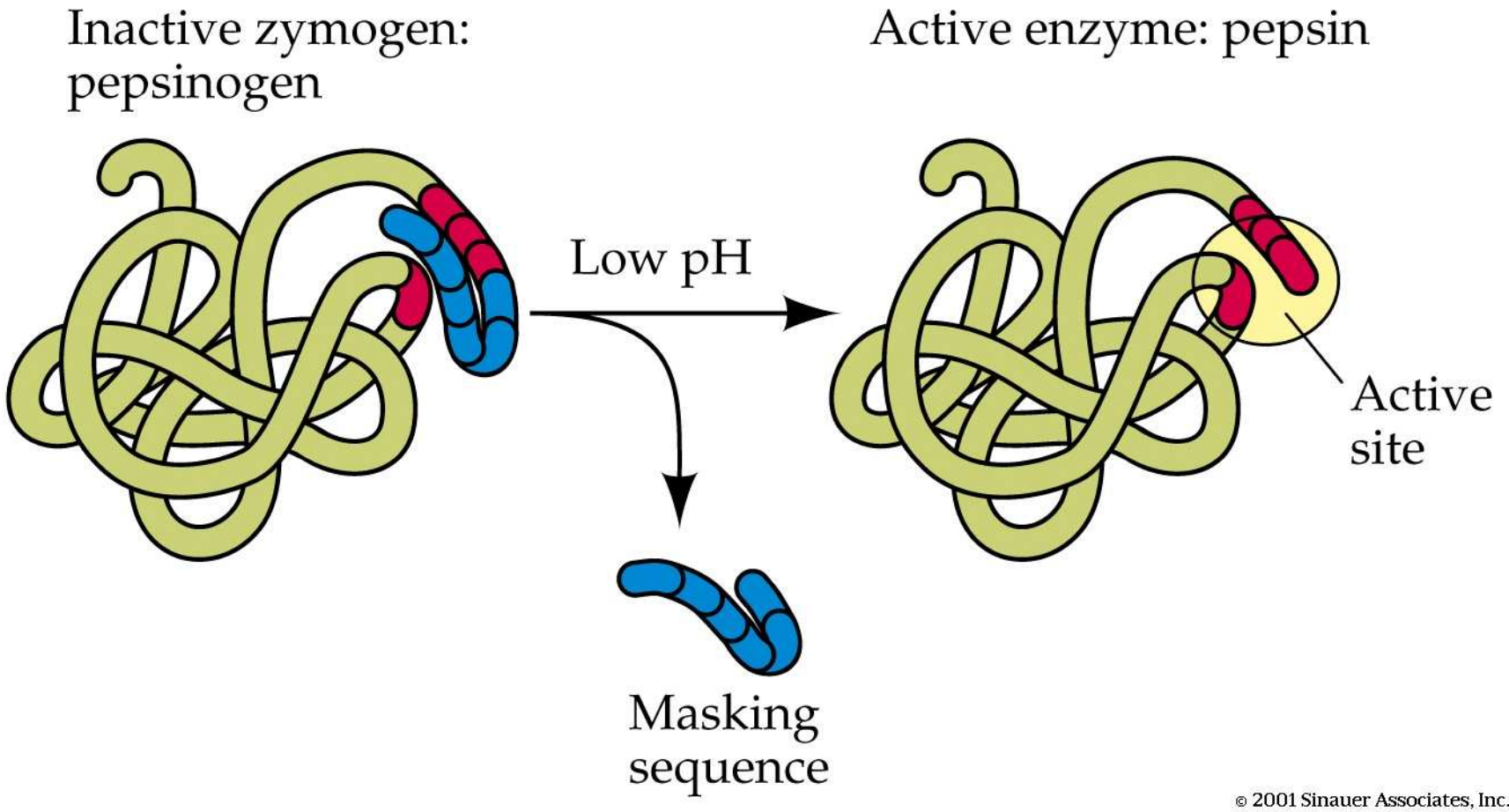
(*b*) Zymogen activation



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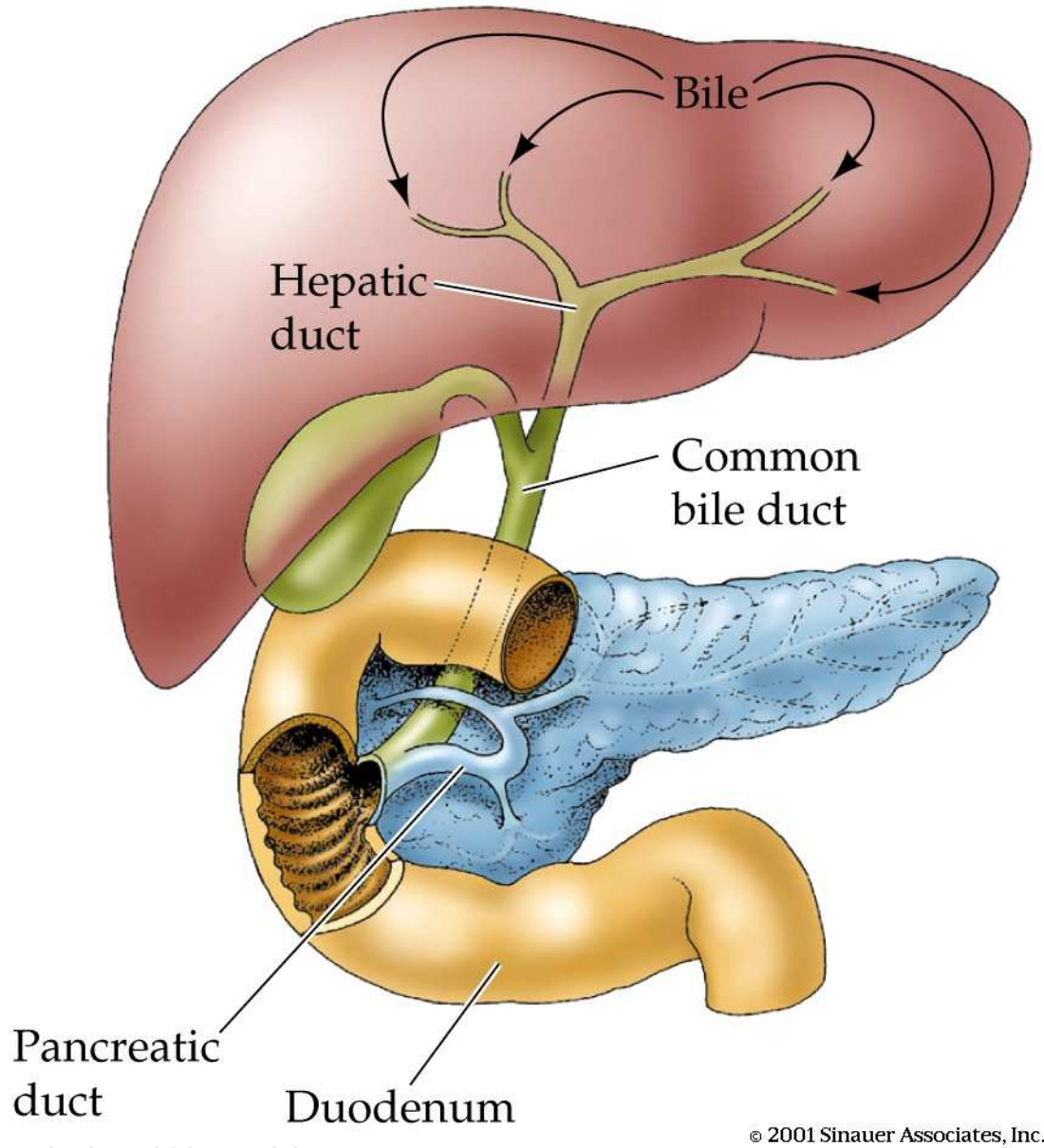

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Sources and Functions of the Major Digestive Enzymes

ENZYME	SOURCE	ACTION	SITE OF ACTION
Salivary amylase	Salivary glands	Starch → Maltose	Mouth
Pepsin	Stomach	Proteins → Peptides; autocatalysis	Stomach
Pancreatic amylase	Pancreas	Starch → Maltose	Small intestine
Lipase	Pancreas	Fats → Fatty acids and glycerol	Small intestine
Nuclease	Pancreas	Nucleic acids → Nucleotides	Small intestine
Trypsin	Pancreas	Proteins → Peptides; activation of zymogens	Small intestine
Chymotrypsin	Pancreas	Proteins → Peptides	Small intestine
Carboxypeptidase	Pancreas	Peptides → Peptides and amino acids	Small intestine
Aminopeptidase	Small intestine	Peptides → Peptides and amino acids	Small intestine
Dipeptidase	Small intestine	Dipeptides → Amino acids	Small intestine
Enterokinase	Small intestine	Trypsinogen → Trypsin	Small intestine
Nuclease	Small intestine	Nucleic acids → Nucleotides	Small intestine
Maltase	Small intestine	Maltose → Glucose	Small intestine
Lactase	Small intestine	Lactose → Galactose and glucose	Small intestine
Sucrase	Small intestine	Sucrose → Fructose and glucose	Small intestine

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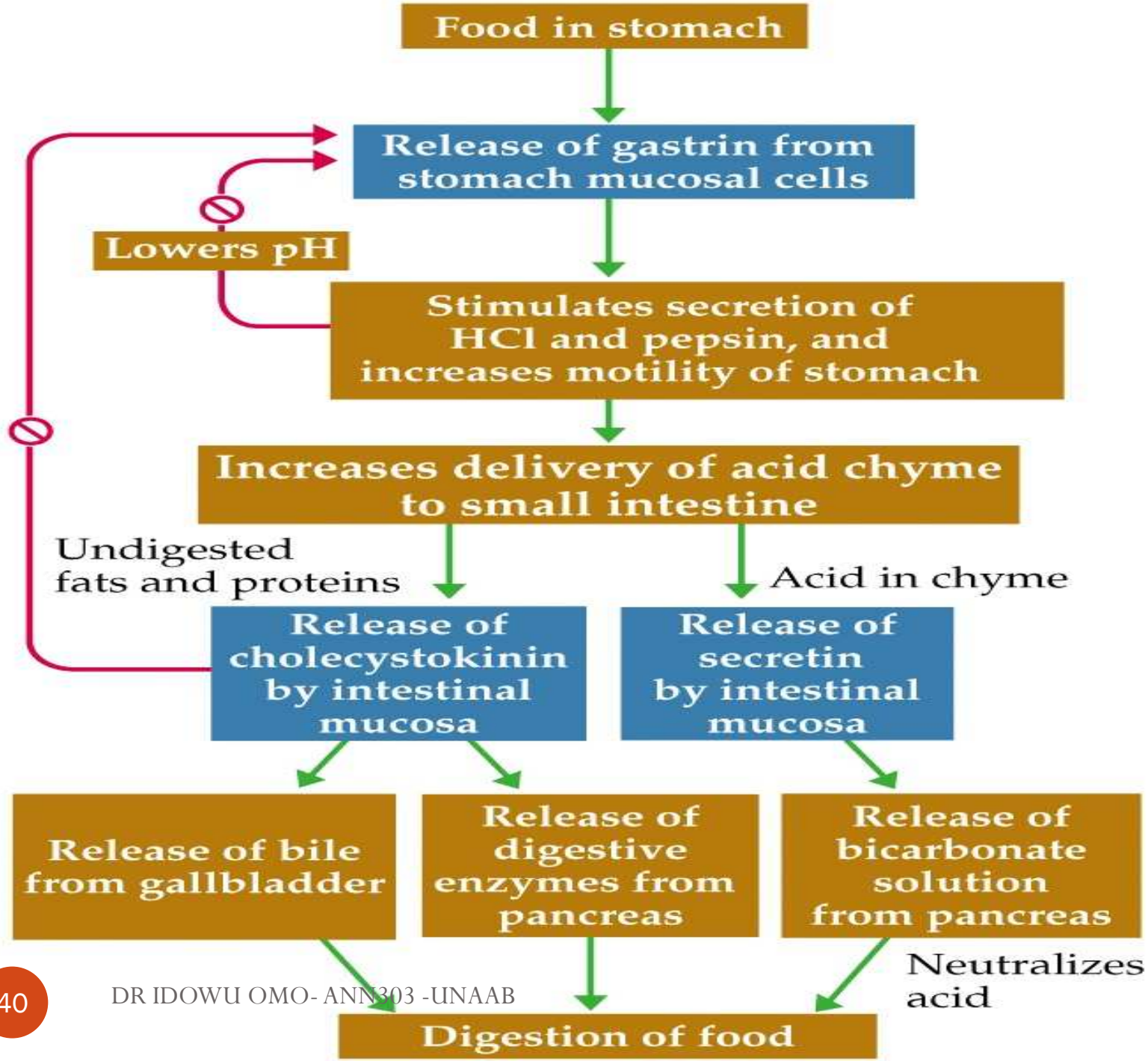
Final enzymatic cleavage of peptides and disaccharides occurs on the cell surfaces of the intestinal mucosa. Amino acids, monosaccharides, and many inorganic ions are absorbed by the microvilli of the mucosal cells.

Often specific carrier proteins in the membranes of these cells transport nutrients into the cells. Sodium cotransport is a common mechanism for actively absorbing nutrient molecules and ions.

Control and Regulation of Digestion

Digestion processes are coordinated and controlled by neural and hormonal mechanisms. Salivation and swallowing are autonomic reflexes. Stomach and small intestine actions are largely controlled by the hormones gastrin, secretin, and cholecystokinin. See Figure

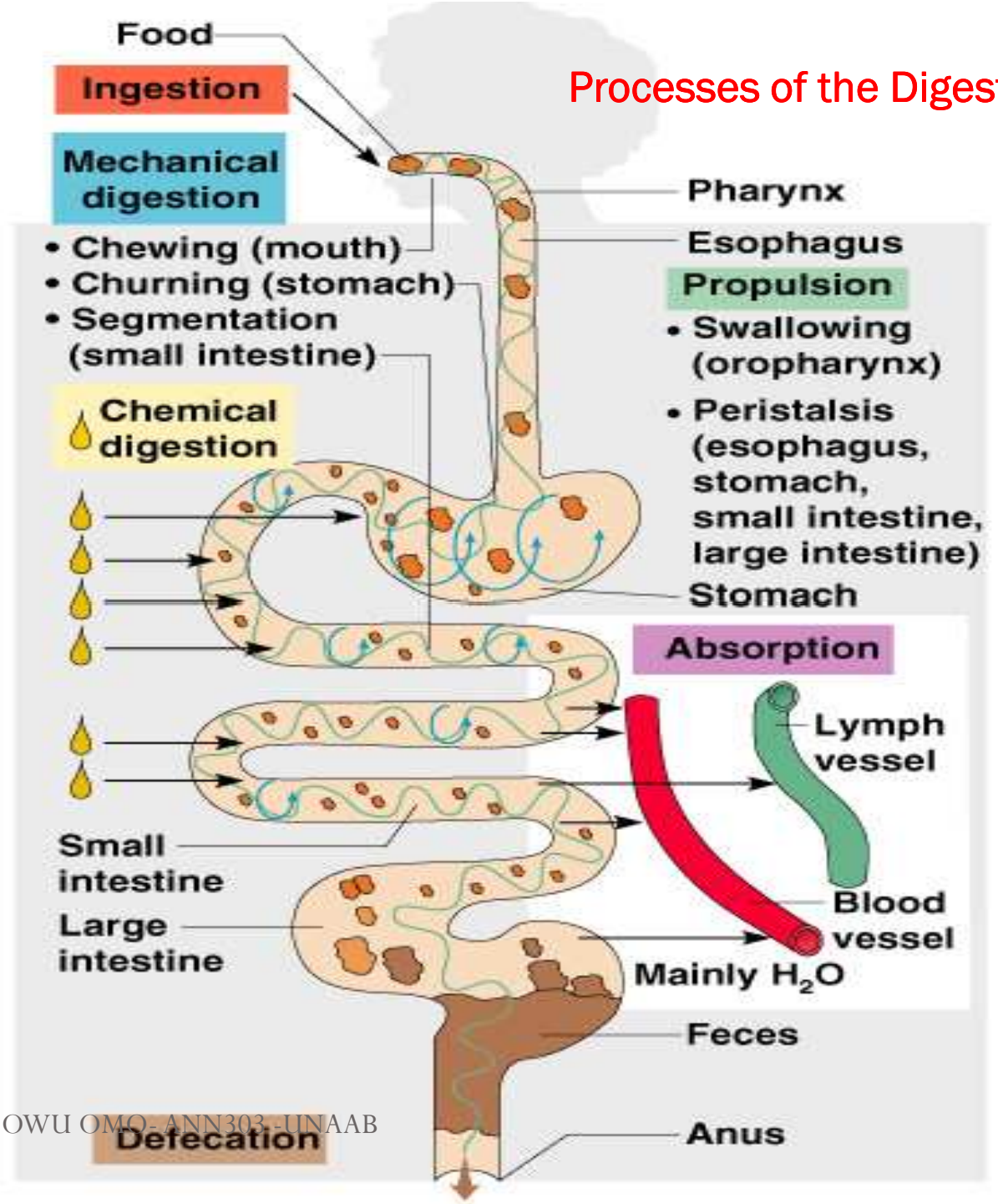

Food in stomach
Release of gastrin from stomach mucosal cells
Lowers pH
Stimulates secretion of HCl and pepsin, and increases motility of stomach
Increases delivery of acid chyme to small intestine
Undigested fats and proteins
Acid in chyme
Release of cholecystokinin by intestinal mucosa
Release of secretin by intestinal mucosa
Release of bile from gallbladder
Release of digestive enzymes from pancreas
Release of bicarbonate solution from pancreas
Neutralizes acid
Digestion of food

Parts and functions of the monogastric mammal digestive system continued…

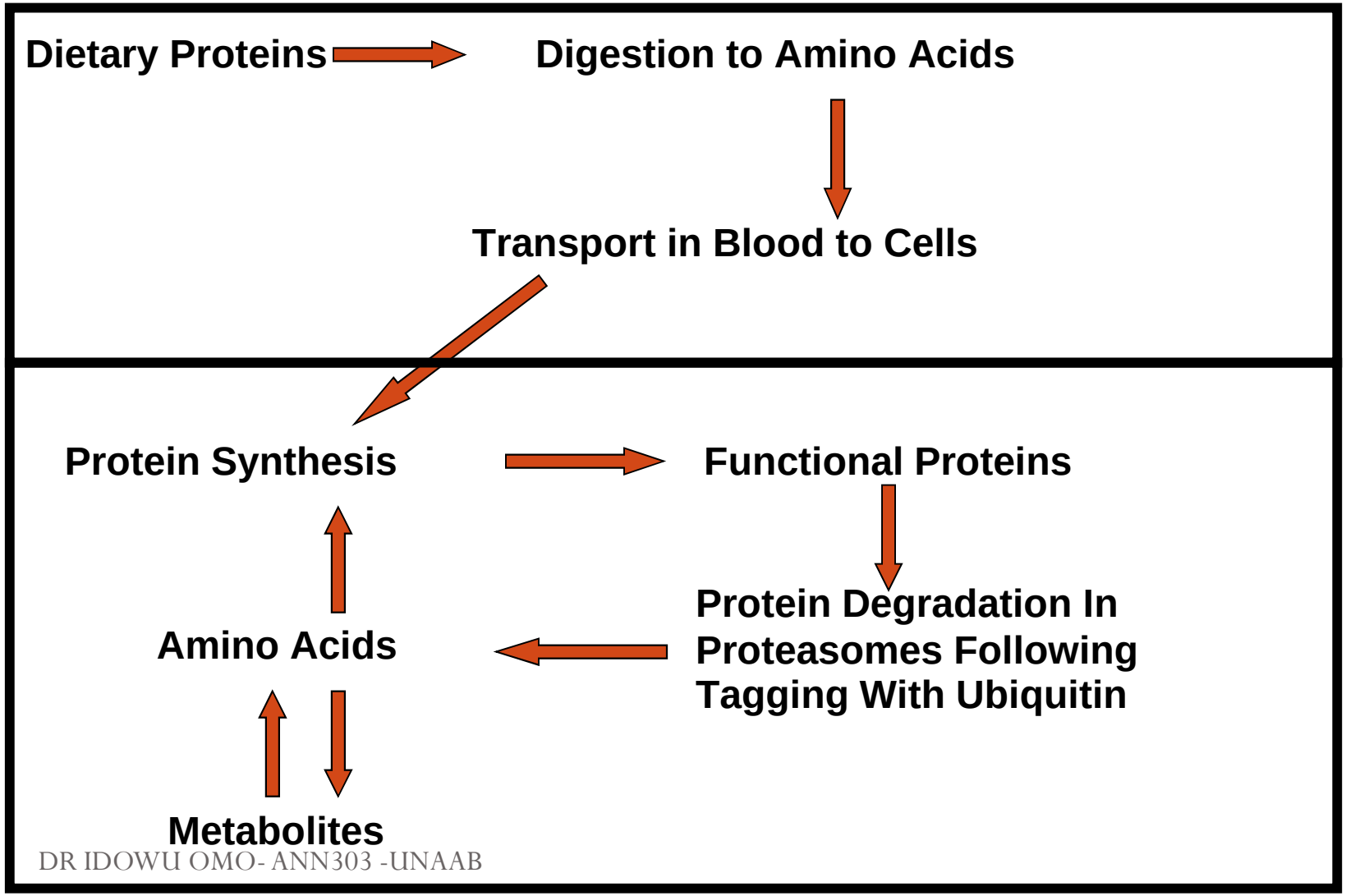
- **Cecum** - essentially non-functioning in many monogastrics. Rabbits and horses have an enlarged cecum that acts like a rumen and is involved with microbial digestion (fermentation)

- **Large Intestine**
  - -bacterial activity
  - -water absorption
  - -waste storage

Processes of the Digestive system



Dynamics of Protein And Amino Acid Metabolism



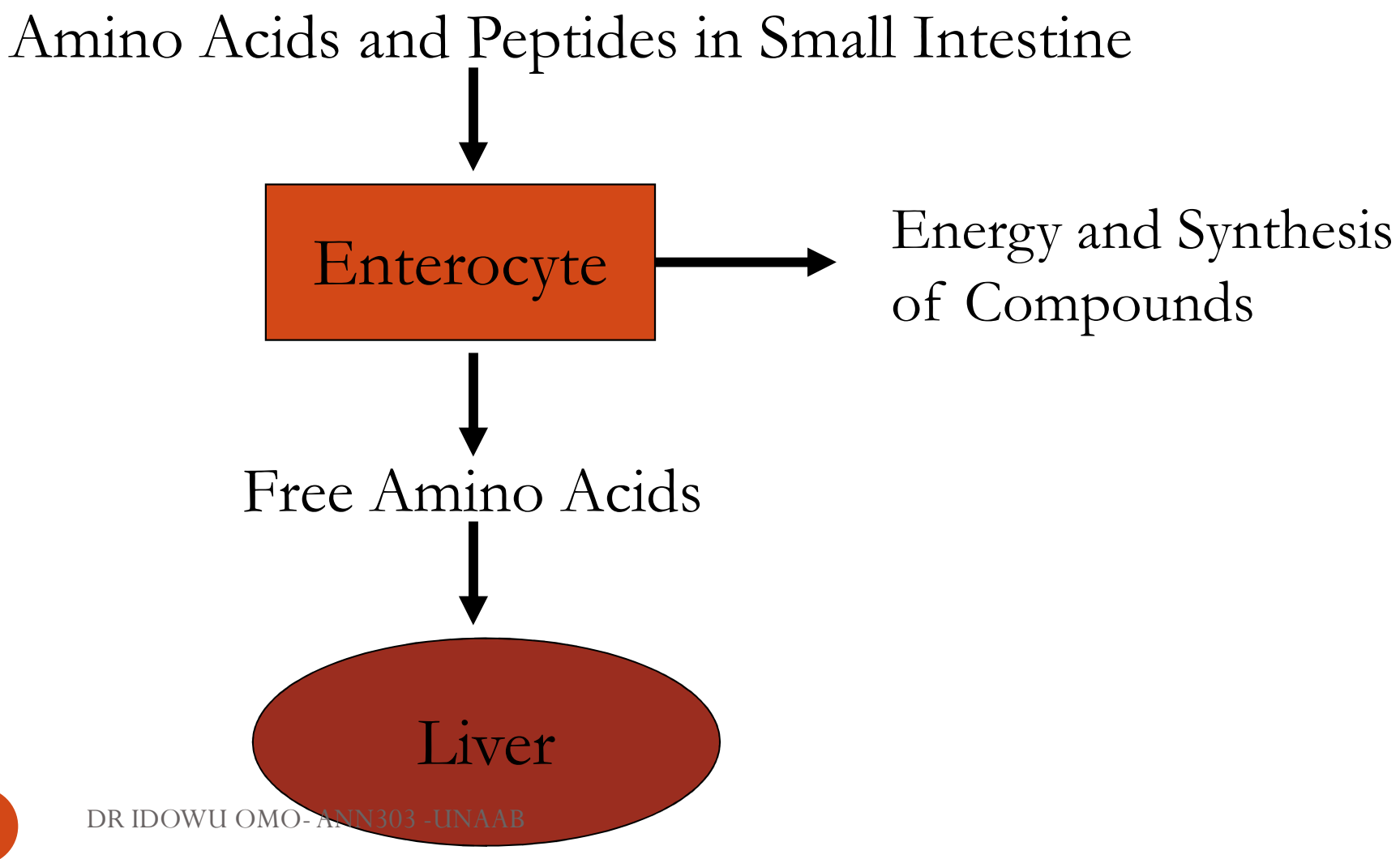
Use of Amino Acids

- Blood
  - Tissue protein synthesis
  - Synthesis of metabolites
    - Enzymes, hormones
  - Deamination or transamination
- Enterocyte
  - Uses amino acids for own needs

Enterocyte Use of Amino Acids

- Energy
  - Primarily glutamine
- Synthesize compounds
  - Apoproteins for lipoprotein formation
  - Digestive enzymes
  - Hormones
  - Other nitrogen-containing compounds

Amino Acid Utilization



Fate of Absorbed Amino Acids

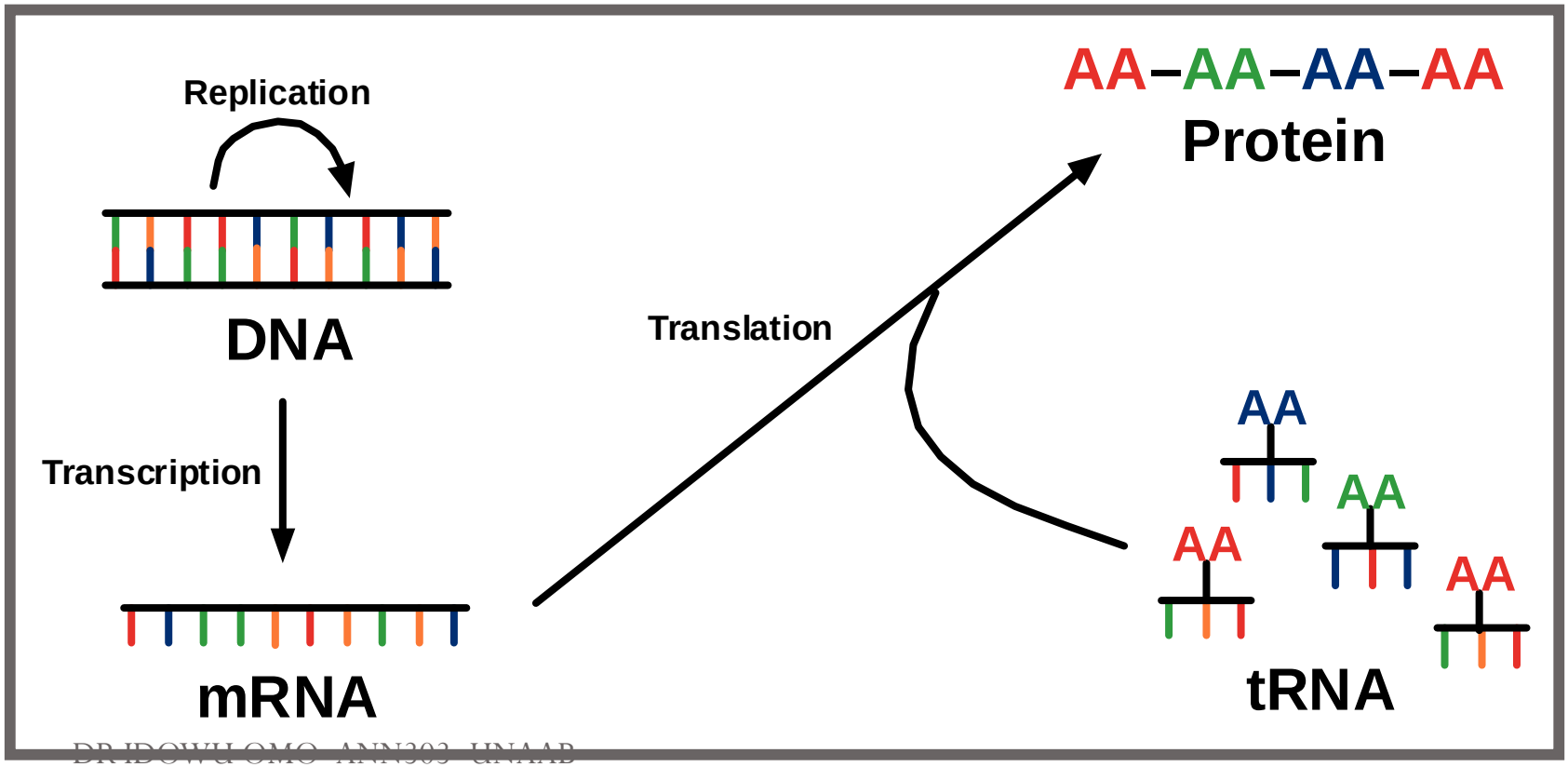
- Three categories
  - Tissue protein synthesis
  - Synthesis of enzymes, hormones, and other metabolites
  - Deamination or transamination

Protein Transport in the Blood

- Amino acids diffuse across the basolateral membrane
  - Enterocytes → portal blood → liver → tissues
  - Transported mostly as free amino acids
- Liver
  - Potential breakdown of many amino acids
    - As needed
  - Synthesis of non-essential amino acids

Protein Metabolism in Cells

- Transport of amino acids from blood into cells
- tRNA immediately 'picks up' amino acid

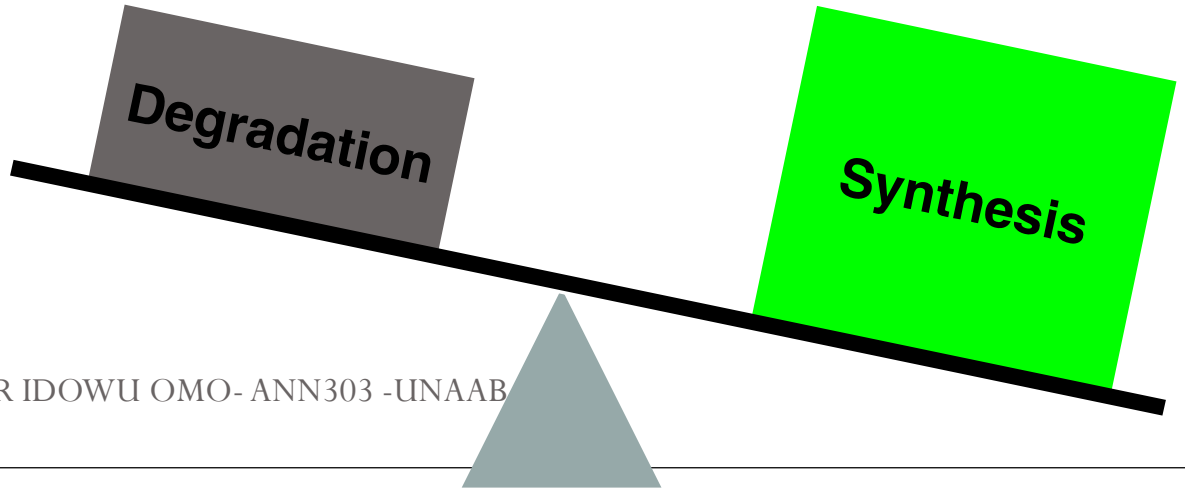


Protein Metabolism in Cells

- No 'storage' of amino acids in cells
  - Synthesis of functional proteins
- Proteins degraded to free amino acids
  - Amino acid concentration in blood is fairly constant

Constant Turnover of Proteins

- **Degradation and re-synthesis of protein**
  - **Some amino acids are degraded and must be replaced through the diet**
    - **'Obligatory losses'**
- **Growth requires that synthesis > degradation**



Protein Synthesis

- On-going, semicontinuous activity in all cells but rate varies greatly between tissues
- Rate is regulated by hormones and supply of amino acids and energy
- Energetically expensive
  - requires about 5 ATP per one peptide bond
- Accounts for about 20% of whole-body energy expenditure

Rate of Protein Synthesis

	Fraction of tissue protein synthesized per day (%/d)	
Tissue	***Pig***	***Steer***
Liver	23	21
Gut	45	39
Muscle	5	2
Muscle is far less "metabolically active" so requires fewer cells to be replaced daily		

Hormonal Regulation

- Insulin
  - Glucose availability to cells increases
  - Protein synthesis increases
- Glucagon
  - Protein synthesis decreases
  - Protein degradation increases

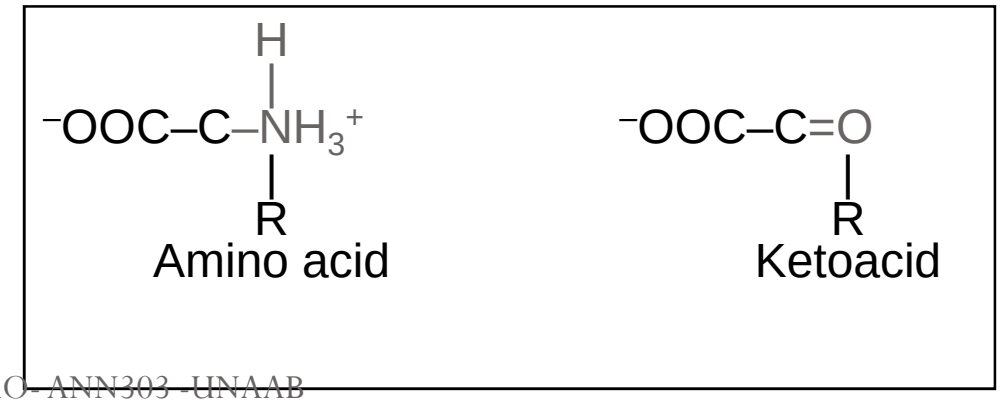
Protein Synthesis

- Protein synthesis of any particular protein cannot proceed without an adequate supply of **all** amino acids that will contribute to the primary structure of that specific protein

Protein Metabolism in Cells

- **All 20 amino acids must be present**
- **If not, must be synthesized in liver**
  - **Synthesis of non-essential amino acids**
    - **Transamination: Transfer of NH_2-group**

Glutamine + ketoacid → Glutamate + amino acid



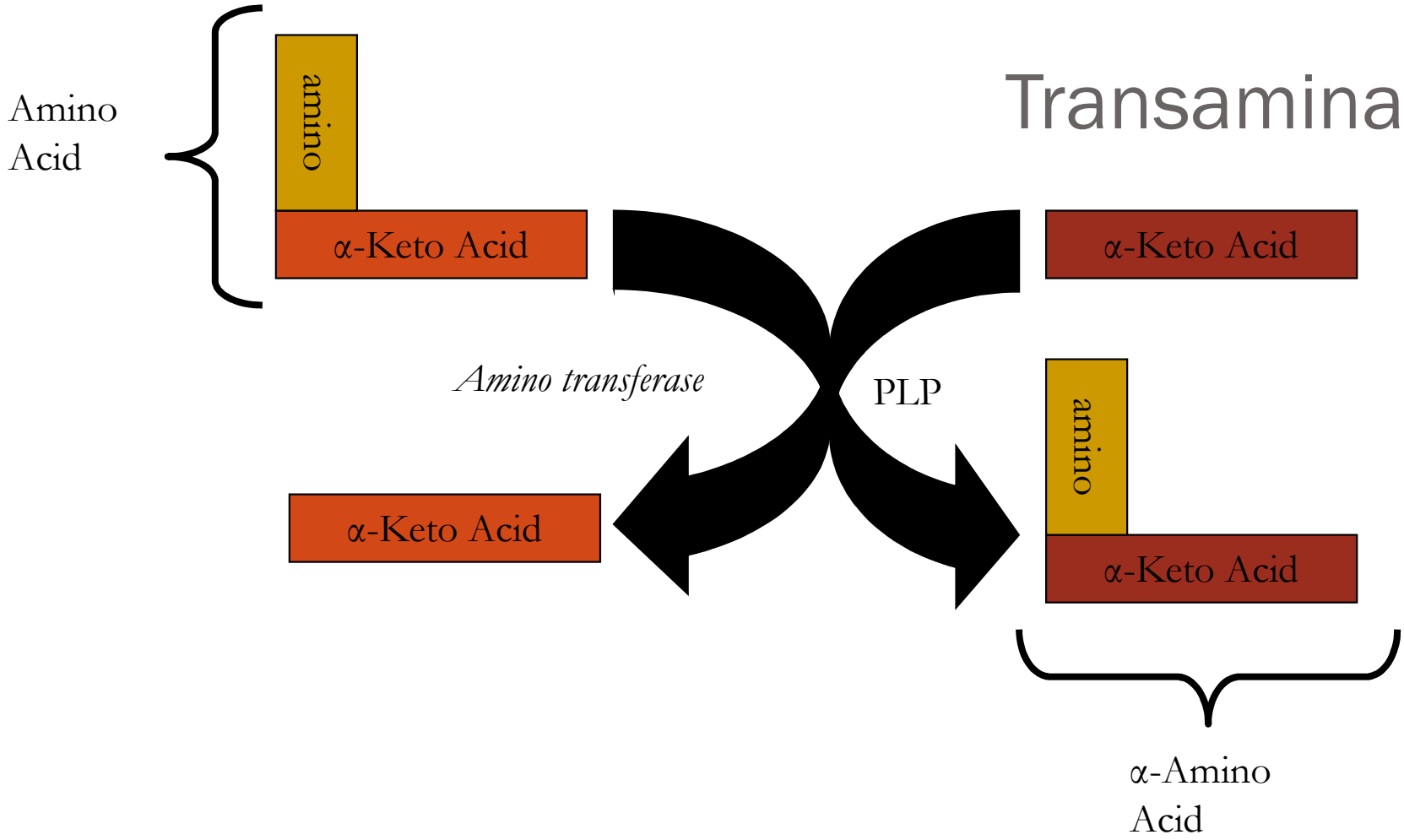
Biosynthesis of Nonessential Amino Acids

- Transamination reactions
  - Allow extensive interconversion between nonessential amino acids
  - Requires vitamin B_6 as a coenzyme

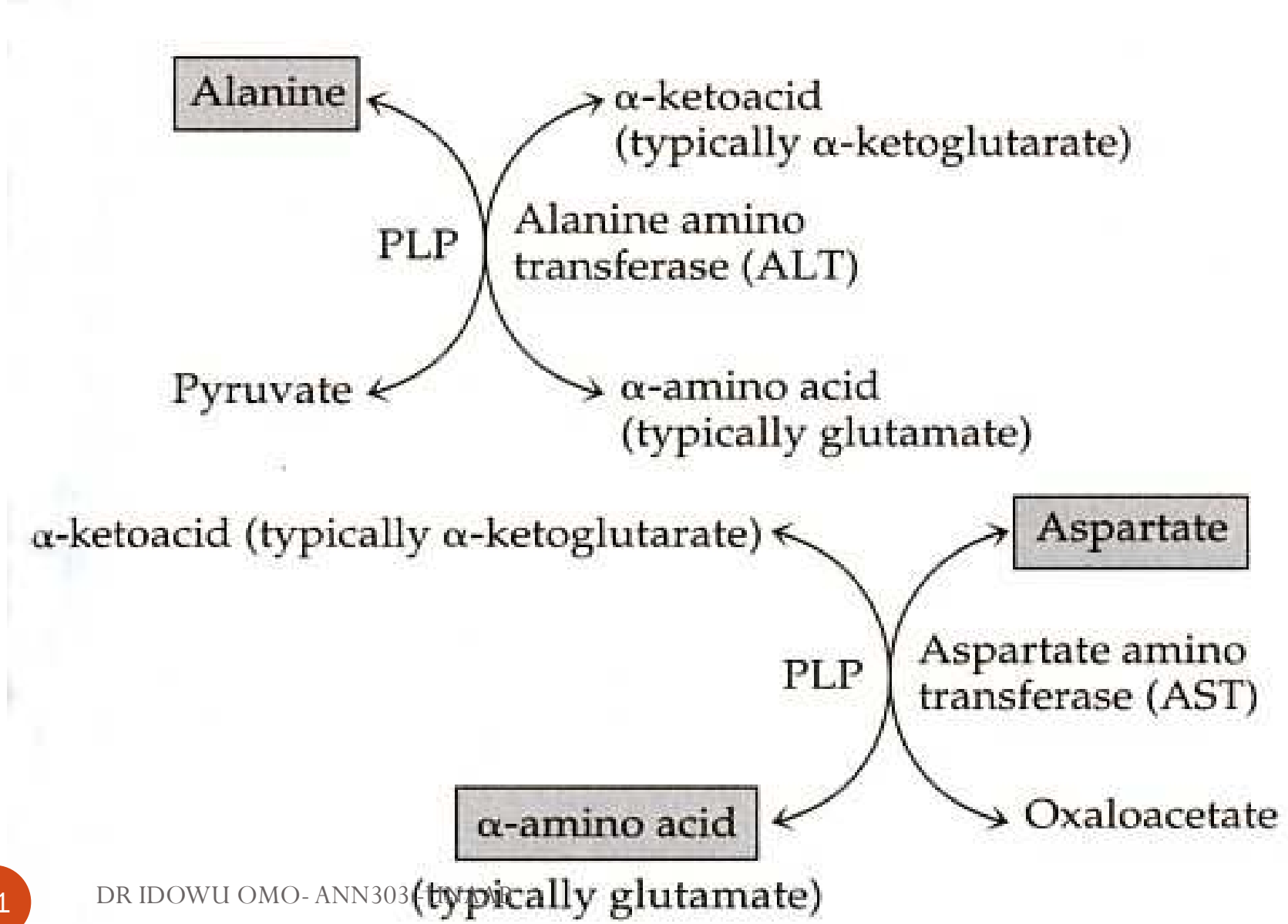
Transamination

- Transfer of amino group from an amino acid to an α-keto acid
- Used to synthesize amino acids as needed
  - Some essential amino acids
    - Not lysine or threonine
  - Must have appropriate α-keto acid in diet
- Requires vitamin B_6 in coenzyme form
  - Pyridoxal phosphate (PLP)
- Catalyzed by amino transferases

Transamination



Transamination



Transaminations

Glutamate
+
Pyruvate

Glutamate-Pyruvate Aminotransferase (Alanine Transferase ALT)

α-Ketoglutarate
+
Alanine

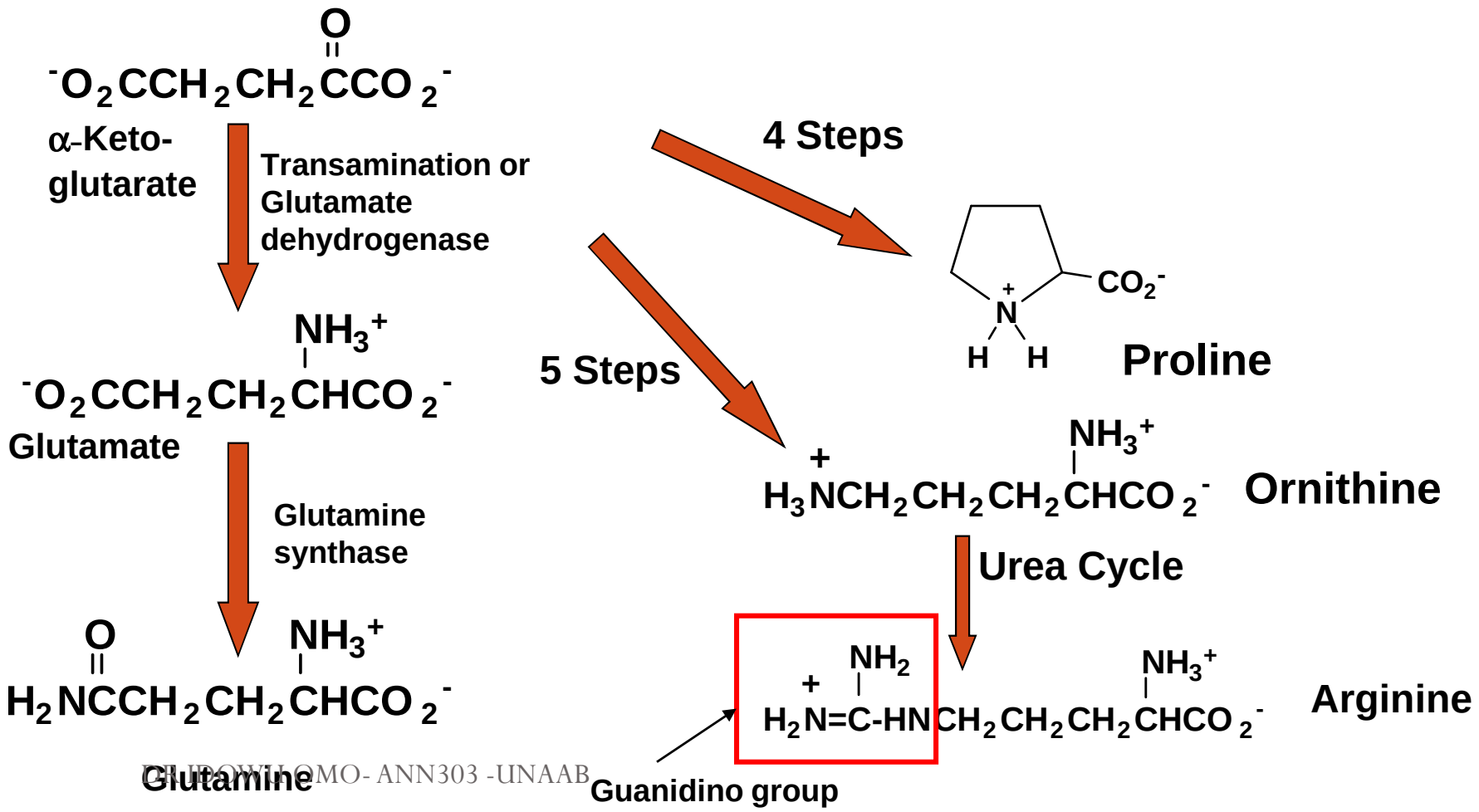
Glutamate
+
Oxaloacetate

Glutamate-Oxaloacetate Aminotransferase (Aspartate Transferase AST)

α-Ketoglutarate
+
Aspartate

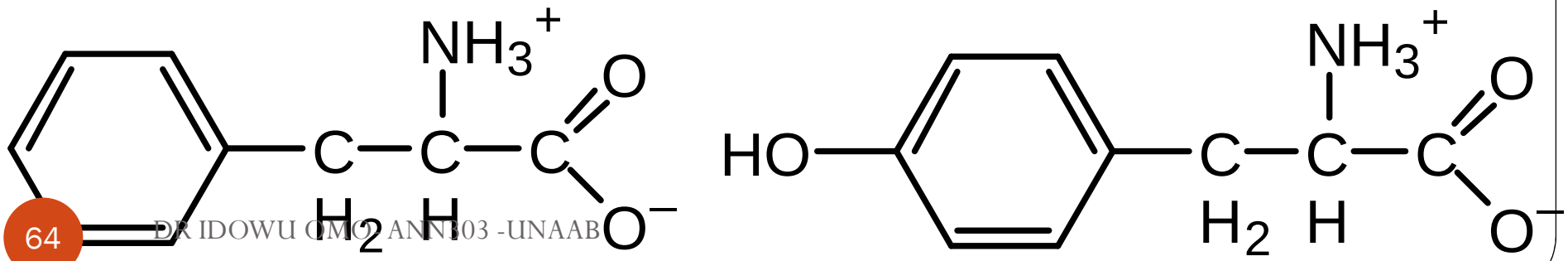
Blood levels of these aminotransferases, also called transaminases, are important indicators of liver disease

Amino Acids Formed From α-Ketoglutarate



Amino Acid Interrelationships

- Methionine can be converted to Cys
  - If too little Cys in diet, Met is converted to Cys and Met becomes deficient
    - Up to 50% of Cys 'requirement' met through Met
- Phe can be converted to Tyr
  - Requirement is typically stated for Phe + Tyr



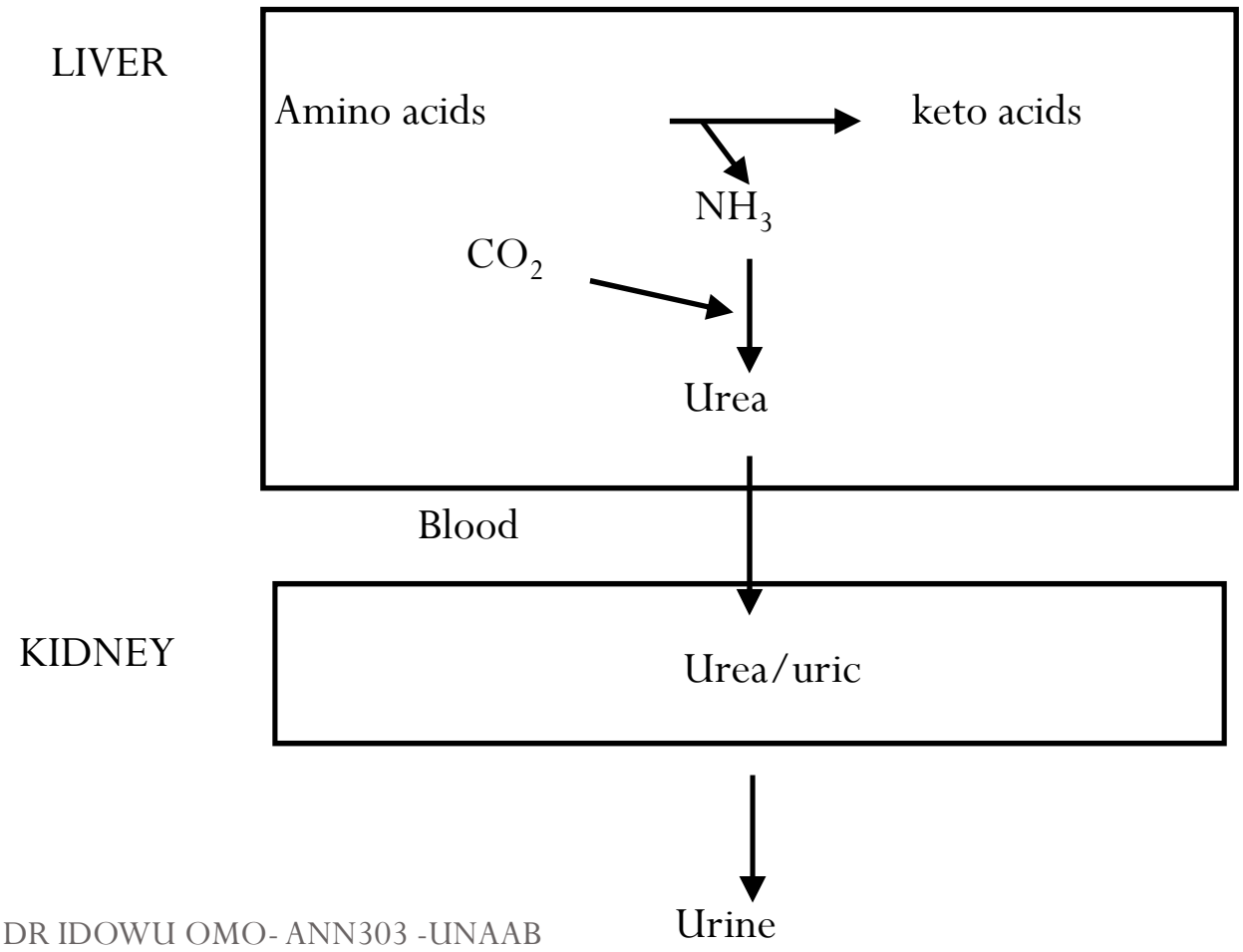
Protein Catabolism

- Occurs when
  - Dietary protein exceeds protein requirements of body
    - Normal situation in true carnivores
    - Abnormal in omnivores and herbivores
  - Composition of absorbed amino acids is unbalanced
  - Gluconeogenesis is increased

Protein Catabolism

- Some net catabolism of body proteins occurs at all times
  - Expressed as *urinary nitrogen excretion*
    - Use the carbon backbone for energy, excrete the nitrogen as urea

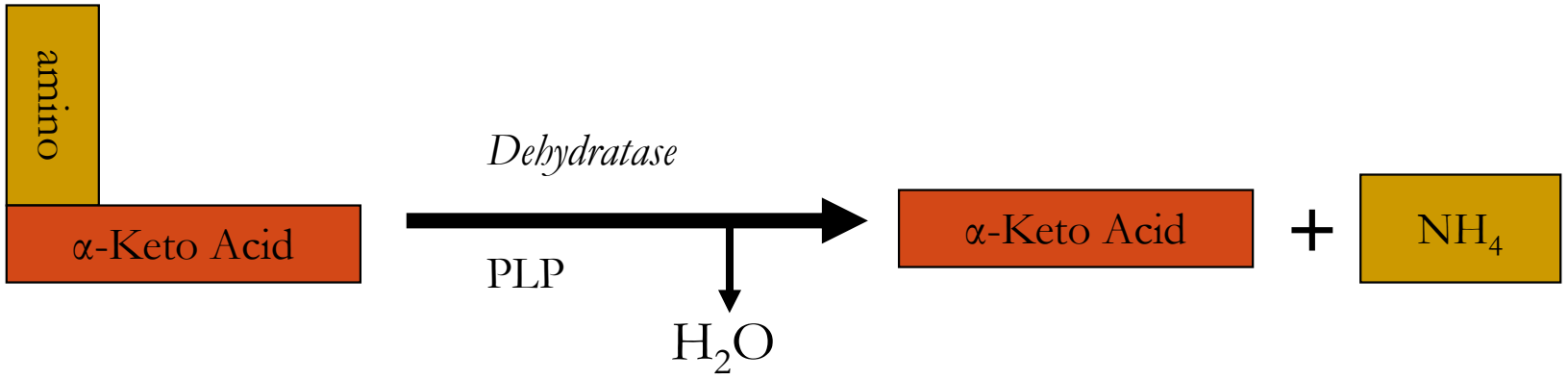
Urinary Nitrogen Excretion

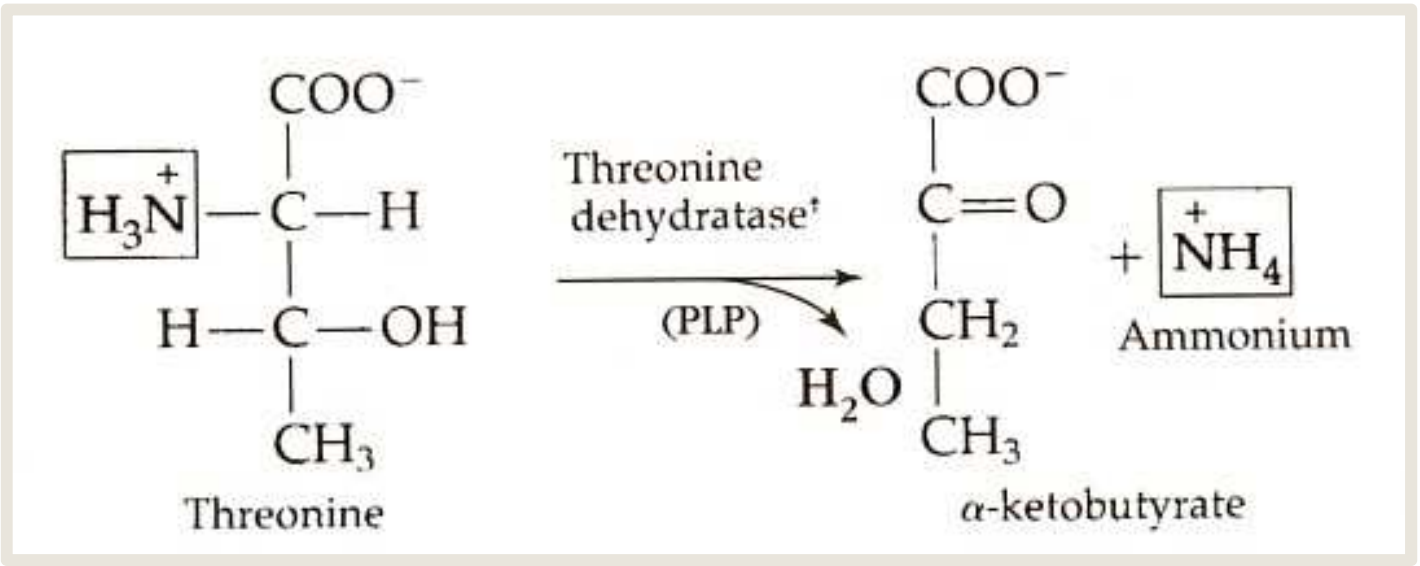


Deamination

- Removal of amino group from an amino acid with no transfer
- Produces ammonia and α-keto acid
  - Ammonia removed by urea/uric cycle
  - α-keto acid is metabolized via several potential pathways
- Pyridoxal phosphate (PLP) required (B_6)

Deamination





Use of Keto Acids for Energy

- **Keto acids can**
  - **Enter the TCA cycle and be broken down to CO_2 and H_2O with release of energy**
  - **Be used for gluconeogenesis**
    - **Some, not all amino acids**
    - **In liver (and kidney)**
  - **Lipogenesis (fatty acid biosynthesis)**
  - **Ketogenesis**
    - **Ketone bodies (acetoacetate, acetyl-CoA)**
    - **Used as energy source in various tissues**

Ketogenic Amino Acids

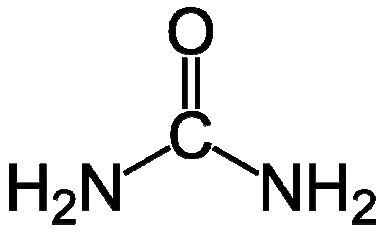
- Leucine and isoleucine
  - Converted to acetoacetate or acetyl CoA in liver
    - Fuel for other tissues

Use of Amino Acids for Energy

- **Not economical**
  - **Energy feeds are less expensive (per kcal) than protein feeds**

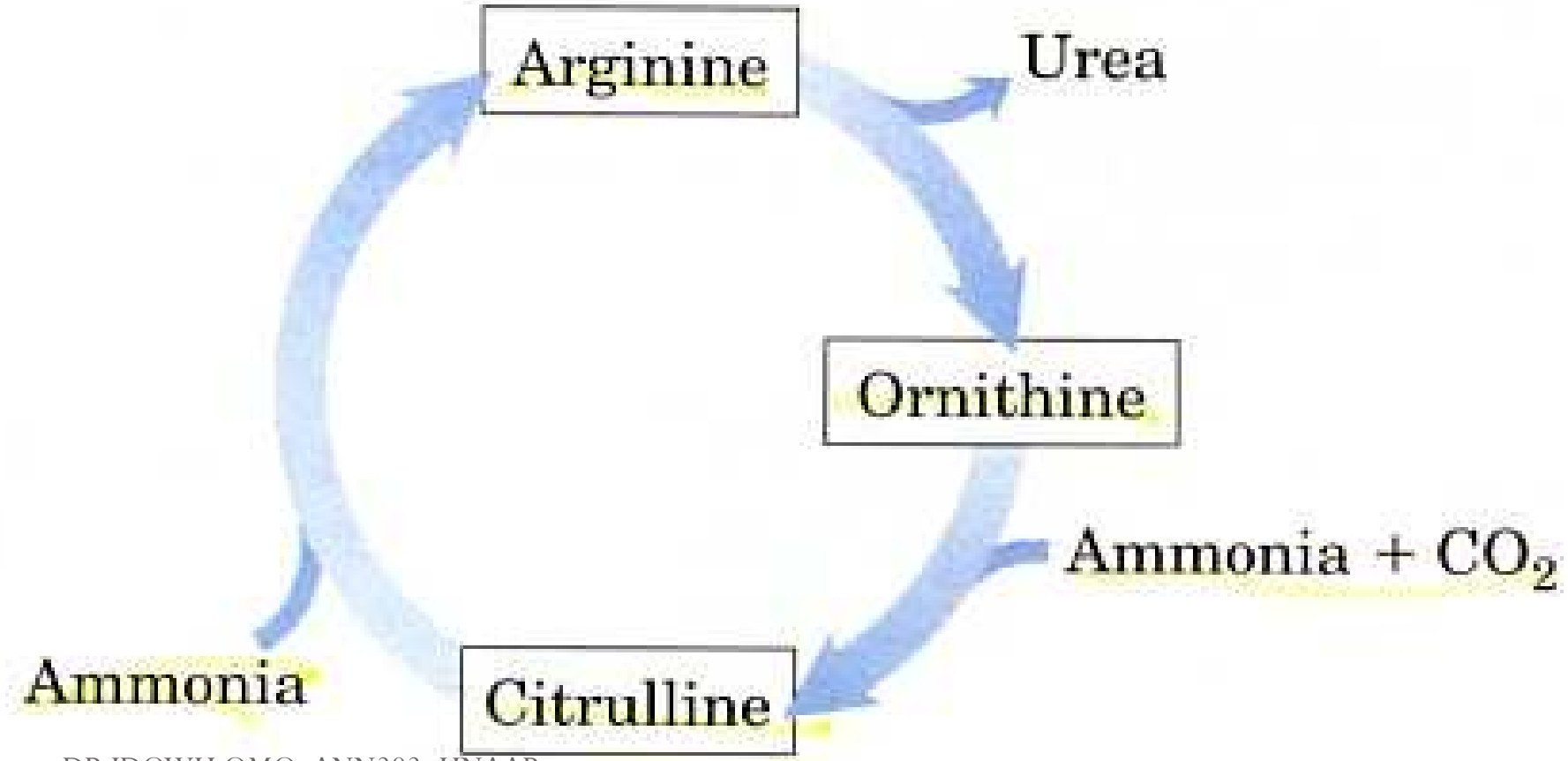
Disposal of NH_3

- NH_3 is very toxic and must be detoxified and excreted from the body
  - Fish: NH_3
  - Mammals: Urea
  - Birds: Uric acid
- Synthesis of uric acid
  - Same pathway as for purines
- Synthesis of urea—the urea cycle
  - Detoxifies NH_3 to urea
  - Synthesizes arginine



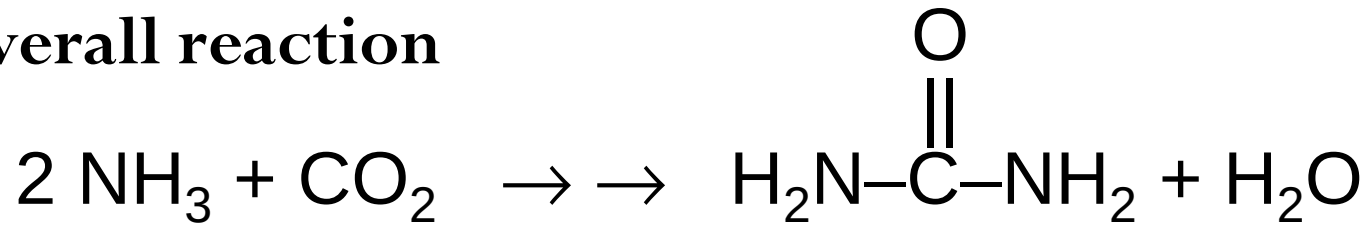
UREA

Urea Cycle



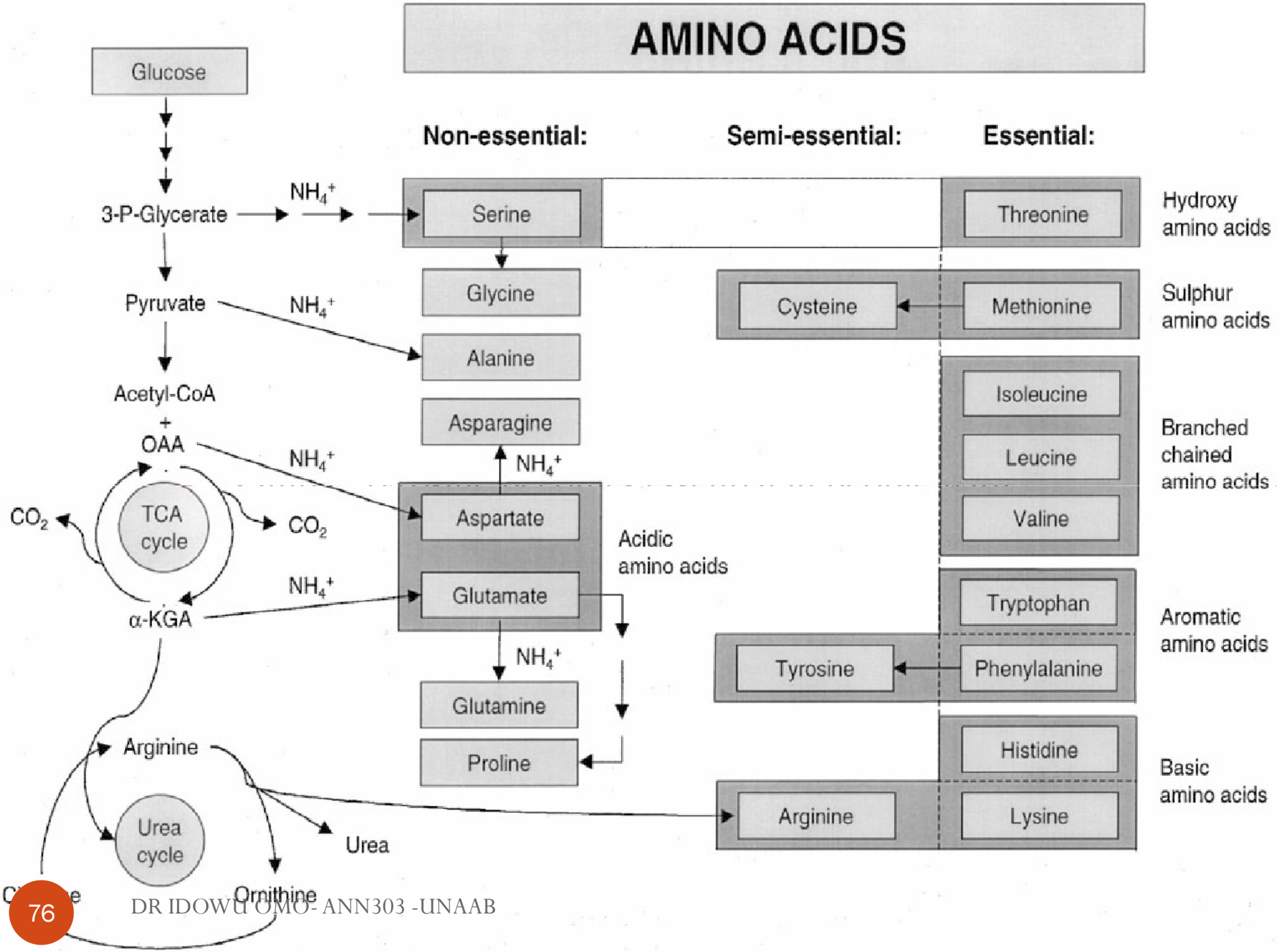
Urea Cycle

- **Overall reaction**



$$2\,NH_3 + CO_2 \rightarrow\rightarrow H_2N\text{–}C(=O)\text{–}NH_2 + H_2O$$

  - Energy required (3 ATP)
  - Urea diffuses from liver cells to body fluids
  - Excreted by the kidneys


AMINO ACIDS
Glucose
3-P-Glycerate
Pyruvate
Acetyl-CoA
+
OAA
TCA cycle
α-KGA
CO_2
CO_2
NH_4^+
NH_4^+
NH_4^+
NH_4^+
NH_4^+
NH_4^+
Arginine
Urea cycle
Urea
Ornithine
Non-essential:
Semi-essential:
Essential:
Serine
Glycine
Alanine
Asparagine
Aspartate
Glutamate
Glutamine
Proline
Acidic amino acids
Threonine
Cysteine
Methionine
Isoleucine
Leucine
Valine
Tryptophan
Tyrosine
Phenylalanine
Histidine
Arginine
Lysine
Hydroxy amino acids
Sulphur amino acids
Branched chained amino acids
Aromatic amino acids
Basic amino acids

Amino Acid Toxicity/Antagonism

- Toxicity/antagonisms are result of dietary imbalances in EAA
- when one EAA is fed in excess it can also increase the requirement for another, structurally-similar EAA
- **toxicity** = overfeeding of one EAA and negative effects not mitigated by increasing other EAA
- **antagonism** = one EAA regulates uptake of another