Protein Digestion and Absorption

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NASPGHAN
Physiology Education Series

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Protein Digestion Objectives

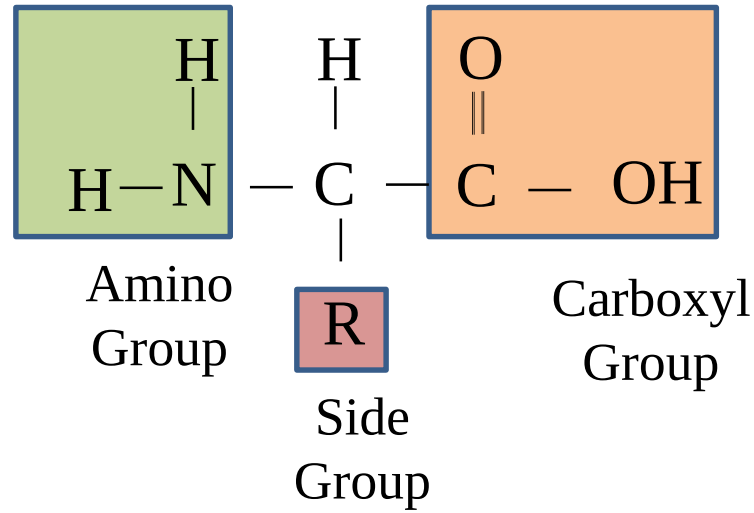
- Understand the structure of protein
- Understand protein digestion and absorption
- Discuss clinical implications

What is Protein?

- Proteins are sequences of amino acids
- There are 20 amino acids
  - 9 are essential amino acids
    - phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, histidine, leucine, lysine
  - 11 are non-essential
  - 4 amino acids are considered conditionally essential
    - arginine, tyrosine, glutamine, and cysteine (glycine, proline and serine sometimes considered conditionally essential)
- Require 0.75g/kg body weight
  - Increased requirements in infants and illness

Amino Acids: Structure

- Consist of a central carbon atom bonded to: a hydrogen, a carboxylic acid, an amino group, and an additional side group that is unique to each amino acid
- The side group creates unique characteristics for each amino acid so they differ in: shape, size, composition, electrical charge, and pH



Amino Acids: Structure

- Amino acids (AA) are linked to form proteins
- Amino acids are joined by PEPTIDE bonds
  - Dipeptide – 2 amino acids
  - Tripeptide – 3 amino acids
  - Oligopeptides – 4-10 amino acids
  - Polypeptide – more than 10 amino acids
- Most proteins in the body and diet are long polypeptides (100s of AA)

Overview of Protein Digestion

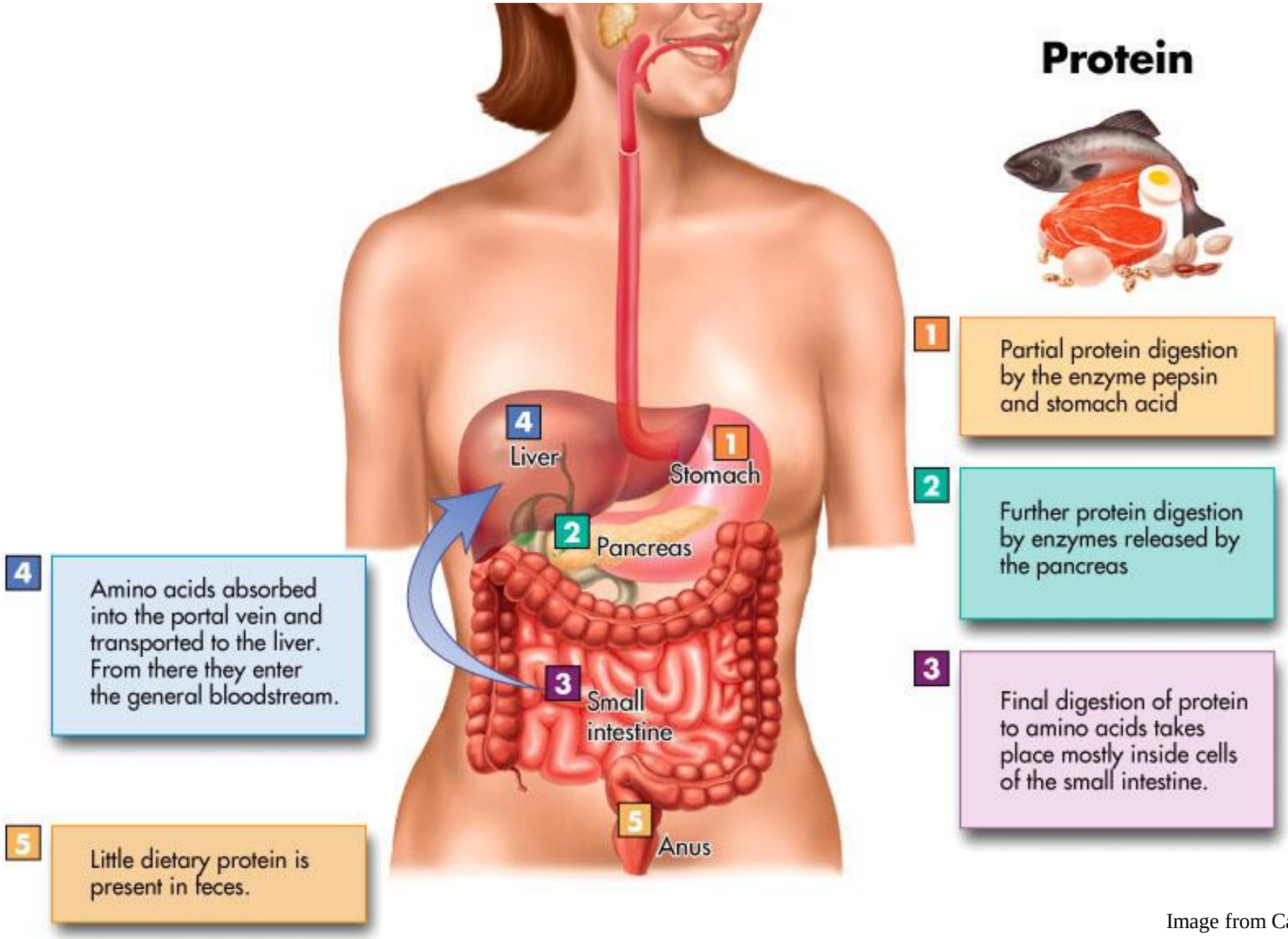


Image from Carolyn Holocroft

Protein Digestion

- Whole proteins are not absorbed
  - Too large to pass through intact cell membranes
- They must be digested into di- and tri-peptides or individual AAs prior to absorption
  - Additional digestion occurs in the cytosol
- Structures of protein are more diverse than carbohydrates
  - Require broad spectrum peptidases and transporters

Protein Digestion - Mouth

- No digestion occurs in the oral cavity or esophagus

Protein Digestion - Stomach

- Gastric phase
  - Gastrin released from G cells
    - Stretch receptors stimulate release
    - AA in stomach stimulate release
  - Parietal cells secrete HCl
    - Gastrin stimulates Parietal cells to secrete HCl
    - HCl denatures proteins - 4^0, 3^0, and 2^0 structures
    - Converts pepsinogen to pepsin

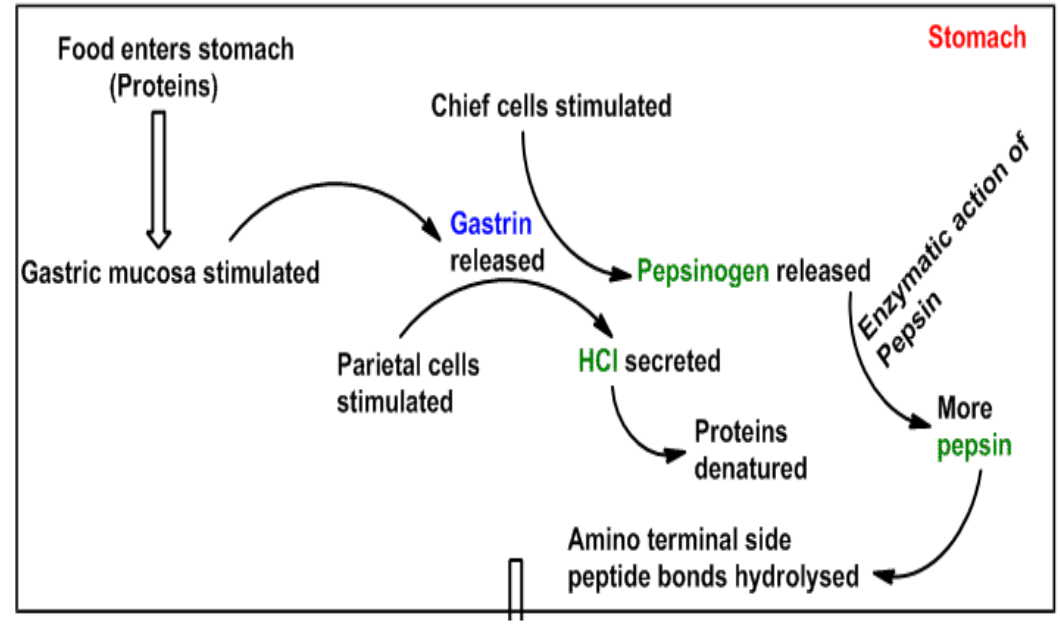


Image from: Sweety Mehta

Protein Digestion - Stomach

- **Chief cells** secrete **Pepsinogen**
  - Stimulated by cephalic vagal input
  - Secretion enhanced
    - Acetylcholine, CCK and gastrin
- Pepsinogen is auto-activated at pH <4 to Pepsin
    - Cleavage of an N-terminal peptide
    - Pepsin can break down collagen
- Pepsin cleaves proteins at large aliphatic or aromatic side groups
  - Completes ~ 10-20% of digestion
- Pepsin is inactivated at pH >4.5
    - Protects intestinal tissues
- Protein leaves stomach as mix of insoluble protein, soluble protein, peptides and amino acids

Protein Digestion - Intestines

- Intestinal phase Overview
  - Majority of proteolysis occurs in the intestines
    - 70% of proteins are converted to oligopeptides
  - Still require terminal action at enterocyte membrane

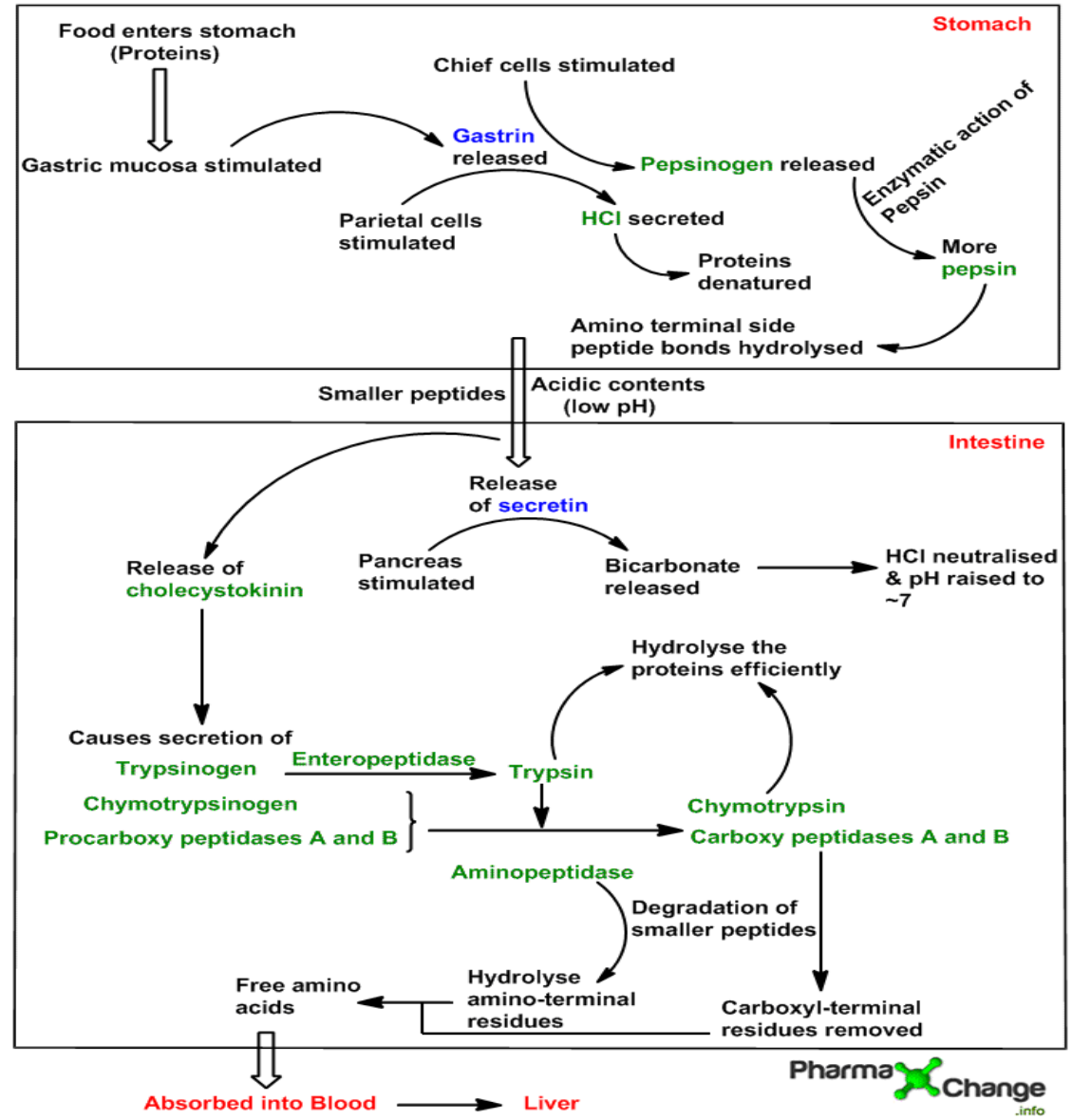


Image from: Sweety Mehta

Protein Digestion – Small Intestine

- Majority of protein digestion occurs within the intestine due to the action of pancreatic proteases.
  - Proteases break down polypeptides into smaller peptides and AA
- Two main forms of pancreatic enzymes
  - Endopeptidase – cleaves internal bonds
  - Ectopeptidase – cleaves AA at C-terminus

Protein Digestion - Intestine

- Endopeptidase: hydrolyze internal peptide bonds:
  - Trypsin (Pancreas)
  - Chymotrypsin (Pancreas)
  - Elastase (Pancreas)
  - Pepsin (Gastric)
- Ectopeptidase: hydrolyzes external peptide bonds:
  - Carboxypeptidase A (Pancreas)
  - Carboxypeptidase B (Pancreas)
  - Aminopeptidase (Pancreas, Brush Border, Cytoplasm)

Pancreatic Proteases

- Pancreatic enzymes stored in acinar cells as pro-enzymes (zymogens)
  - Trypsinogen $\longrightarrow$ Trypsin
  - Chymotrypsinogen $\longrightarrow$ Chymotrypsin
  - Procarboxypeptidase A and B $\longrightarrow$ Carboxypeptidase A and B
  - Proelastase $\longrightarrow$ Elastase

Protein Digestion - Pancreas

- Zymogens are converted to active form in the intestine
  - Trypsinogen $\xrightarrow{\text{Enterokinase/ Trypsin}}$ Trypsin
    - Endopeptidase
      - Cleaves on carbonyl side of Lysine & Arginine
  - Chymotrypsinogen $\xrightarrow{\text{Trypsin}}$ Chymotrypsin
    - Endopeptidase
      - Cleaves carboxy terminal tyrosine, phenylalanine, tryptophan, methionine, and leucine
  - Procarboxypeptidase A/B $\xrightarrow{\text{Trypsin}}$ Carboxypeptidase
    - Ectopeptidase
      - Removes carboxy terminal residues

Protein Digestion - Intestine

- Large peptides from gastric proteolysis are sequentially cleaved in the small intestine
  - Endopeptidases – cleave in the middle of peptide chains
    - Trypsin
    - Chymotrypsin
    - Elastase
  - Yield shorter chains with neutral or basic AA at C-terminus
  - Carboxypeptidase A and Carboxypeptidase B then act
    - Carboxypeptidase A act on neutral AA
    - Carboxypeptidase B acts on basic AA

Protein Digestion - Trypsin Inhibitors

- Small proteins or peptides
- Present in plants, organs, and fluids
  - Soybeans, peas, beans, wheat
  - Pancreas, colostrum
- Decrease activity of trypsin
  - *Decrease all other proteases activated by trypsin*
- Inactivated by heat

Protein Digestion - Mucosal

- Intraluminal degradation of proteins by gastric and pancreatic enzymes is incomplete
- Brush border hydrolysis necessary
- Diversity of substrates requires diverse hydrolyases
  - Membrane bound on villi
  - Not present in crypt cells
  - Produce free AA and small peptides

Protein Digestion - Mucosal

Small intestine

(brush border)

- Aminopeptidases
  - Cleave at N-terminal AA
- Dipeptidases
  - Cleave dipeptides
- Carboxypeptidases
  - Cleaves at the carboxy-terminal

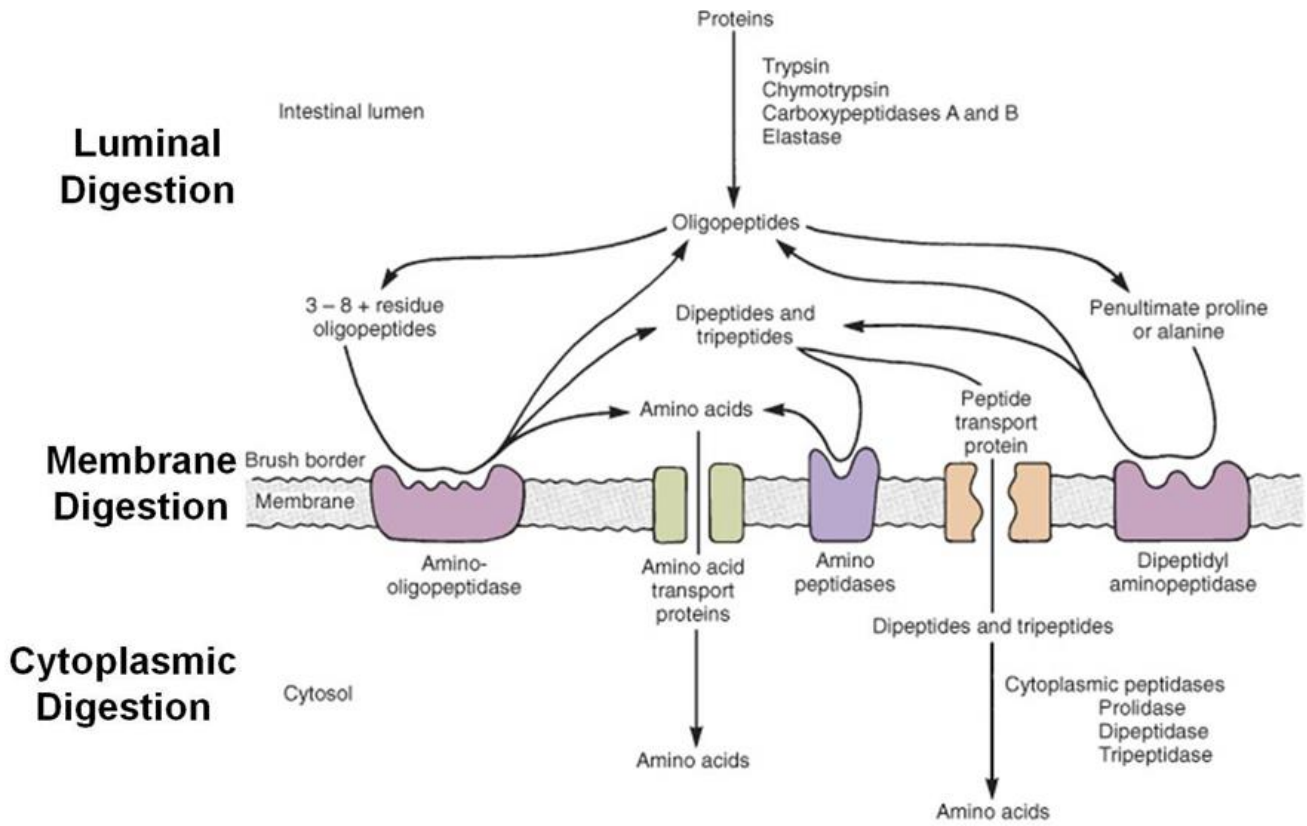


Image from John Yaw-Jong Tsai

Protein Digestion - Cytosol

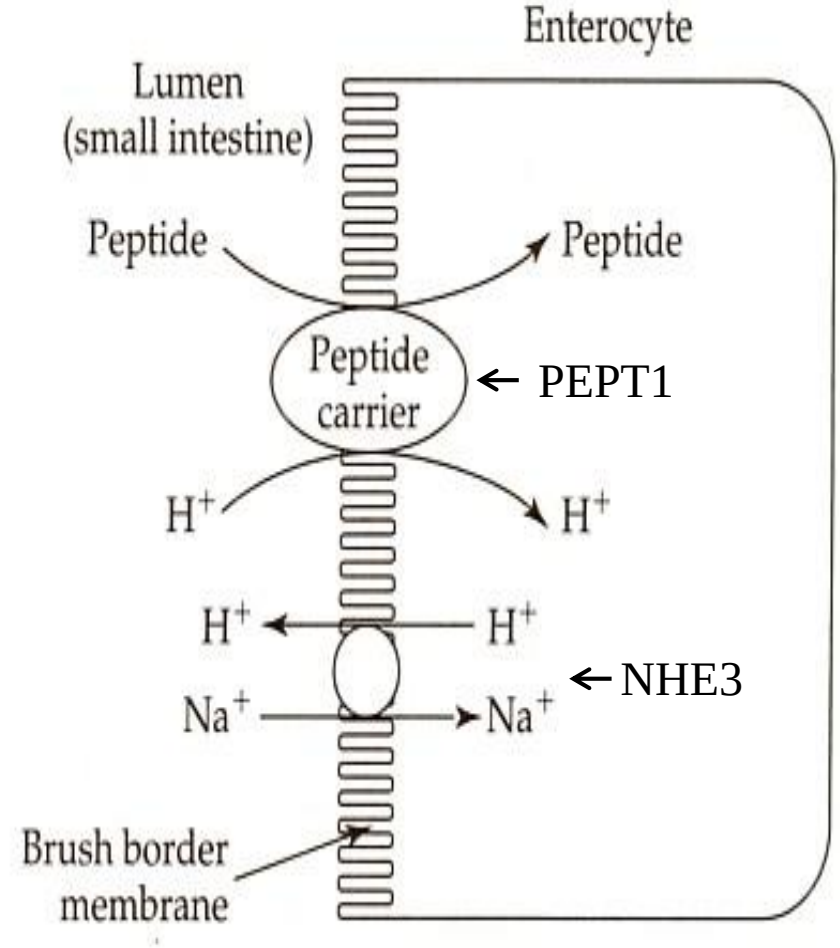
- The vast majority of di- and tri-peptides are digested into amino acids by cytoplasmic peptidases.
- Peptidases
  - Cleave N-terminal AAs

Protein Absorption Overview

- Most protein absorption takes place in the duodenum and jejunum
  - Tripeptides, Dipeptides and AA are absorbed
  - There is minimal absorption of peptides longer than three amino acids
- Most AA are absorbed into the bloodstream, but some remain in the enterocytes and are used to support the cells
- >99% of protein enters the bloodstream as amino acids

Protein Absorption - Peptides

- Essentially no absorption of peptides longer than three amino acids
- Di- and tri-peptides are more rapidly absorbed than free amino acids due to PEPT1
  - Active transporter – PEPT1
    - Coupled to sodium-hydrogen exchanger (NHE3)
    - Accommodates various sizes and charges
- Di- and tri-peptides are digested into amino acids by cytoplasmic peptidases



Groff & Gropper, 2000

Amino Acid Absorption/Transport - Enterocyte

Transporter	Substrate	Ion dependence	Disease
SLC1A1	Anionic amino acids	Na^+, K^+, H^+	Dicarboxylic aciduria
SLC1A5	Ala,Ser,Cys,Gln,Asn	Na^+	
SLC6A6	Taurine	Na^+, Cl^-	
SLC6A14	Neutral and cationic amino acids	Na^+, Cl^-	
SLC6A19	Neutral amino acids	Na^+	Hartnup
SLC6A20	Imino acids	Na^+, Cl^-	
SLC7A9/SLC3A1#	Neutral and cationic amino acids, cysteine	None	Cystinuria
SLC36A1	Small neutral amino acids	H^+	

- heterodimer

Barrett, Gastrointestinal Physiology, 201

Protein Absorption - Amino Acids

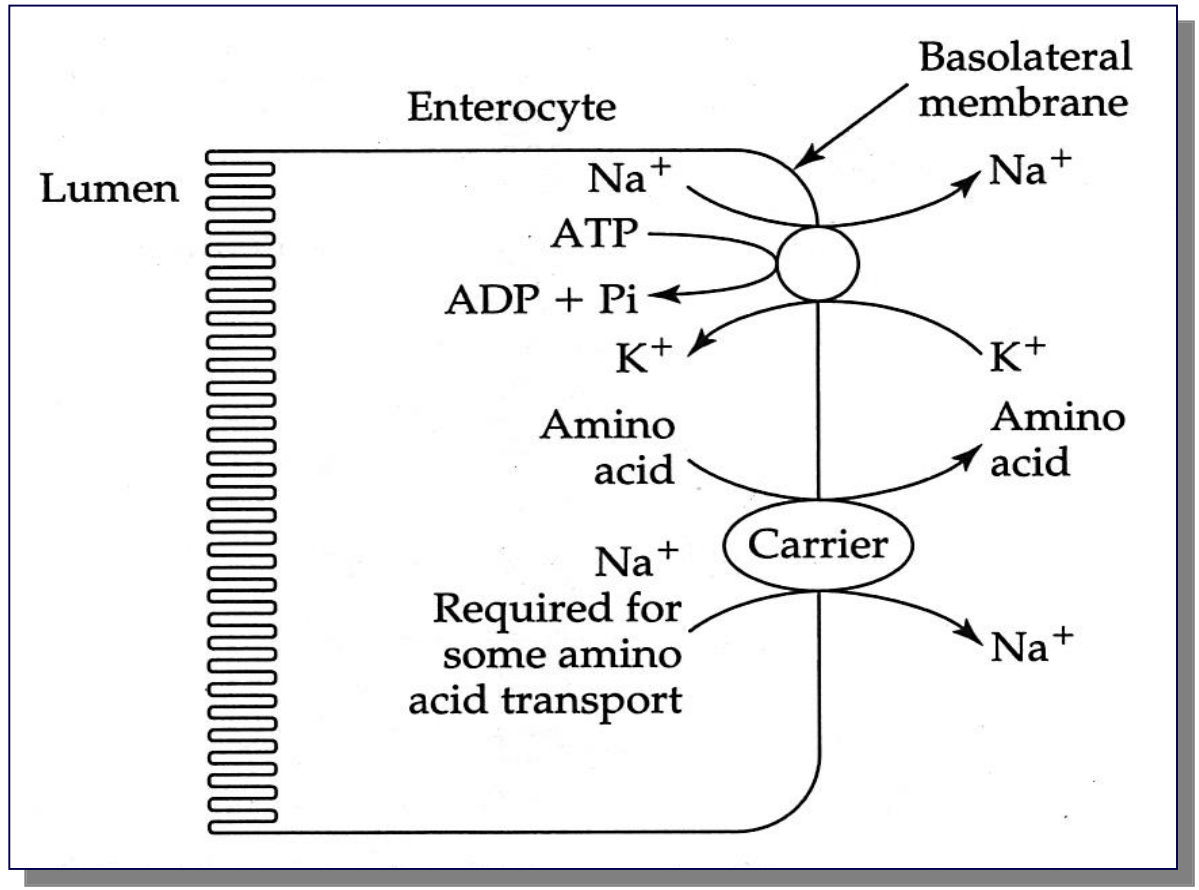
- The Na^+ dependent transporters mechanism
  - Transporter binds amino acids only **after** binding sodium
  - Conformational change occurs - dumping Na^+ and the amino acid into the cytoplasm
  - The transporter then re-orients back to its original form allow transport of the next amino acid
  - Absorption of amino acids is dependent on the electrochemical gradient of Na^+ across the epithelium

Protein Absorption - Amino Acids

- There are 7 transporters of free amino acids in the brush border
- Mechanism varies
  - Na^+-independent carriers (2)
    - Proton co-transport
    - Facilitated diffusion
  - Na^+-dependent (5)
    - Carriers are coupled to Na+ concentration gradient

Protein Transport - Enterocyte

- The basolateral membrane of the enterocyte contains transporters which export amino acids from the cell into the blood
  - Diffusion
  - Both Na+ dependent and independent carriers
- Only free amino acids absorbed into blood



Groff & Gropper, 2000

Protein Absorption - Intact Proteins

- Absorption of intact proteins occurs rarely
- Shortly after birth, neonates can absorb intact proteins
- Very few proteins can get through the gauntlet of soluble (lumen) and membrane-bound proteases intact
- “Normal” enterocytes do not have the transporters needed to carry proteins across the plasma membrane and they can’t permeate tight junctions

Clinical Correlation

- Hartnup Disease
  - Abnormal transport of neutral AA (ie – tryptophan)
    - Mutation in SCL6A19 transporter
  - Clinical variability
    - No symptoms to rash or neurologic symptoms
    - Due to lack of nicotinamide (tryptophan metabolite)
  - Diagnosis
    - High levels of neutral AA in the urine
  - Treatment – supplement with nicotinamide

Clinical Correlation

- Cystinuria
  - Decreased intestinal absorption of dibasic AA (lysine, arginine, cystine)
    - Defect in SLC3A1 transporter
  - Presentation
    - Kidney stones – accounts for 10%
  - Diagnosis
    - Elevated urine cystine
  - Treatment
    - Hydration
    - Limit dietary sodium and methionine

Protein Digestion - Summary

- Proteins are digested into amino acids and di and tri peptides in the stomach and small intestine
  - The vast majority of digestion is due to pancreatic enzymes
    - Enterokinase plays a key role in pancreatic enzyme activation
  - A small percentage of digestion occurs following absorption into the cytosol
- Absorption is complex
  - Several different transporters
  - Several different mechanisms

Review Questions

- There are 9 essential amino acids. Please list them –
-

Review Question

- The intestinal phase is responsible for the bulk of protein digestion. What enzymes are active in the intestines?
  - HCL and Pepsinogen
  - Endopeptidase and Ectopeptidase
  - Pepsin and Trypsin
  - Trypsin, Chymotrypsin and Proelastase

Questions?

Please send any questions or comments to

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