

Malabsorption: The laboratory's contribution to the unsettled gut

Chemistry Seminar
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This speaker has nothing to disclose.

+ An unsettled stomach

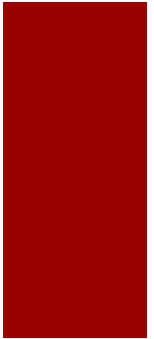
- 9 month old girl
- Skin rash → antibiotics
- Diarrhea next day
- Switch to soy-based formula, then protein hydrolysate
- Hospitalized



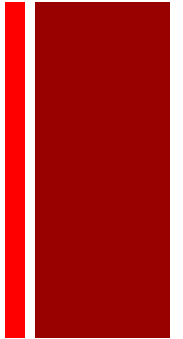
+ Learning Objectives

By the end of the session participants should be able to:

- Recall the anatomic location and physiological processes of digestive organs
- List several causes of malabsorption
- Suggest appropriate laboratory tests to aid in the evaluation of suspected malabsorption



+ Outline



- **Review of the digestive system**
 - Gastrointestinal anatomy and physiology
 - Mechanisms of nutrient breakdown
 - Nutrient absorption
 - Causes of abnormal function

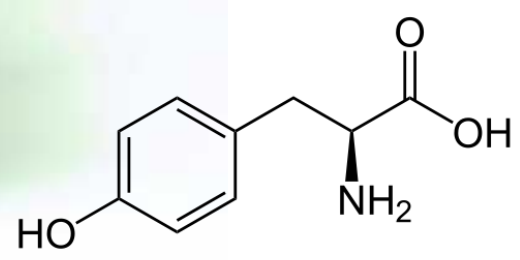
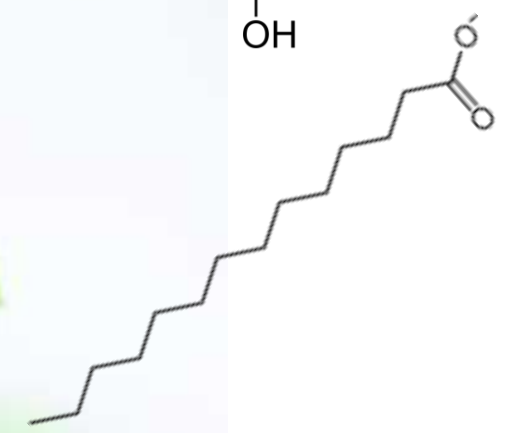
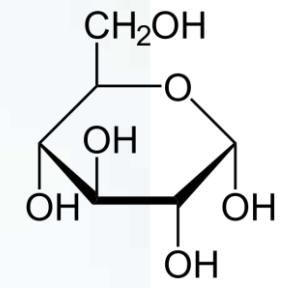
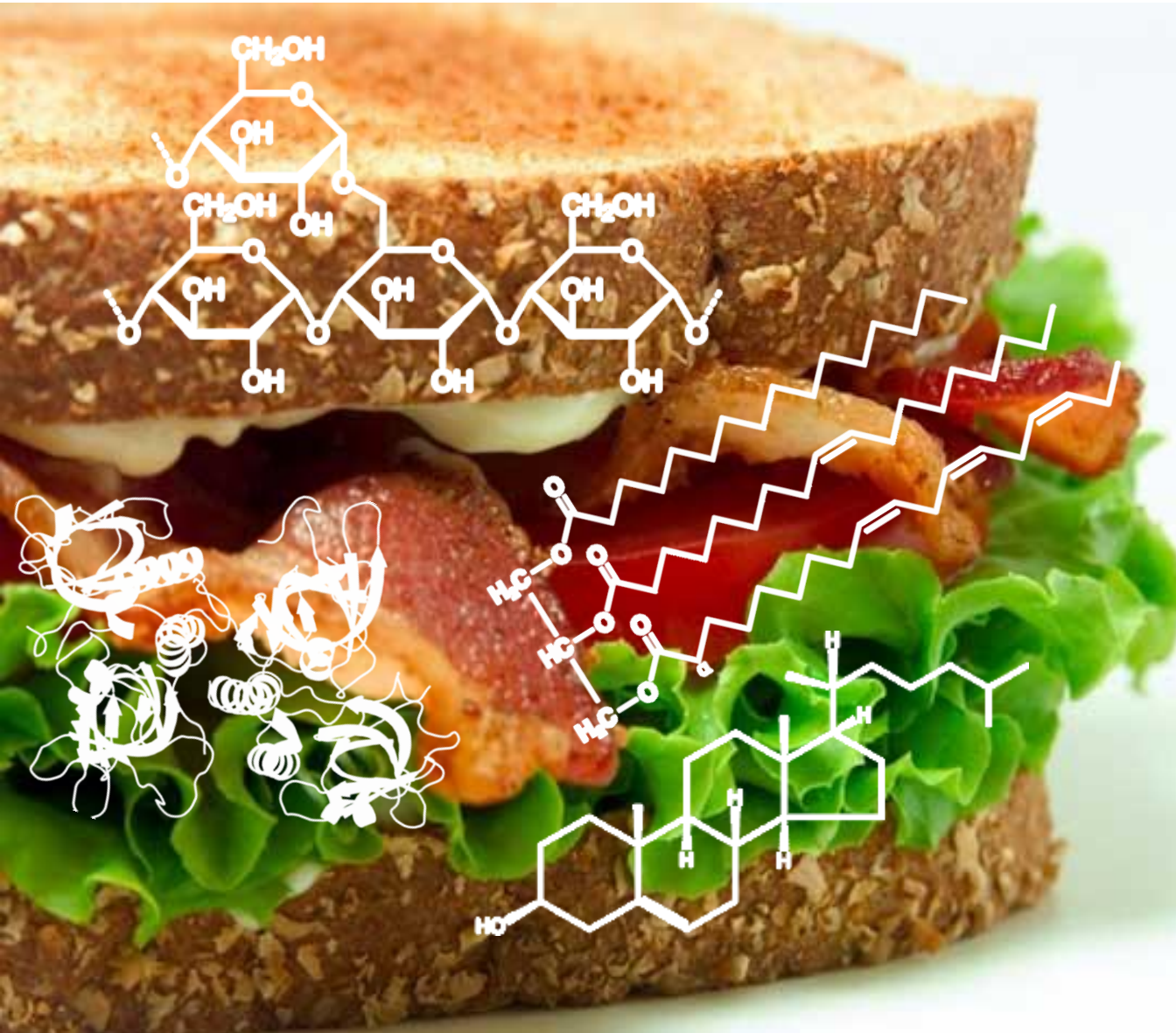
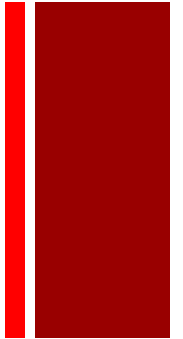
- **Malabsorption**
 - Symptoms
 - Laboratory evaluation
 - Management

+ Digestion and Absorption

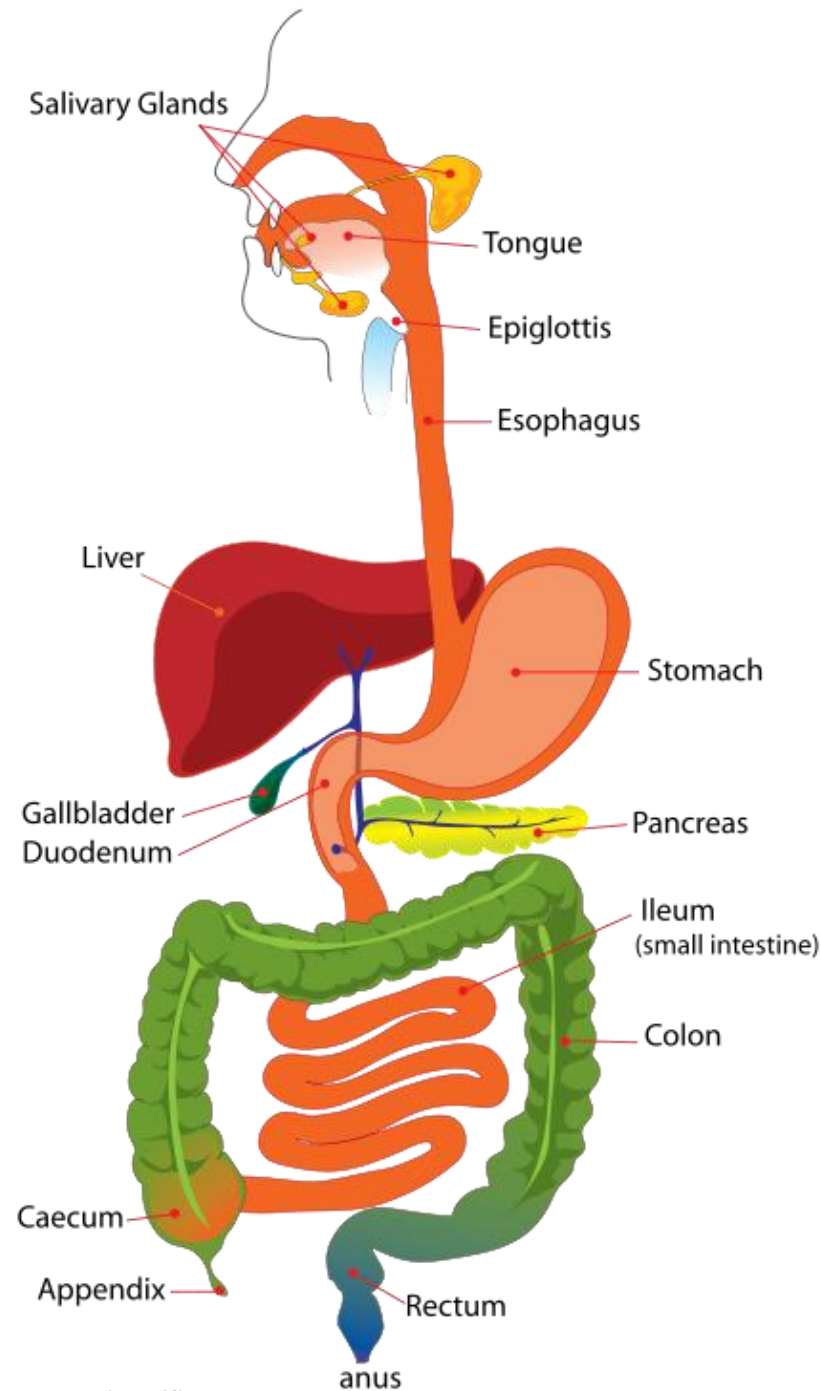
- The process by which nutrients are consumed, broken down, absorbed and transported to other parts of the body
- Mechanical, chemical processes
- Three phases:
 - Luminal → breakdown, solubilization
 - Mucosal → movement of nutrients into GI cells
 - Transport → distribution of nutrients throughout the body
- Allows food nutrients to be utilized for energy and growth



+ Format of Nutrients

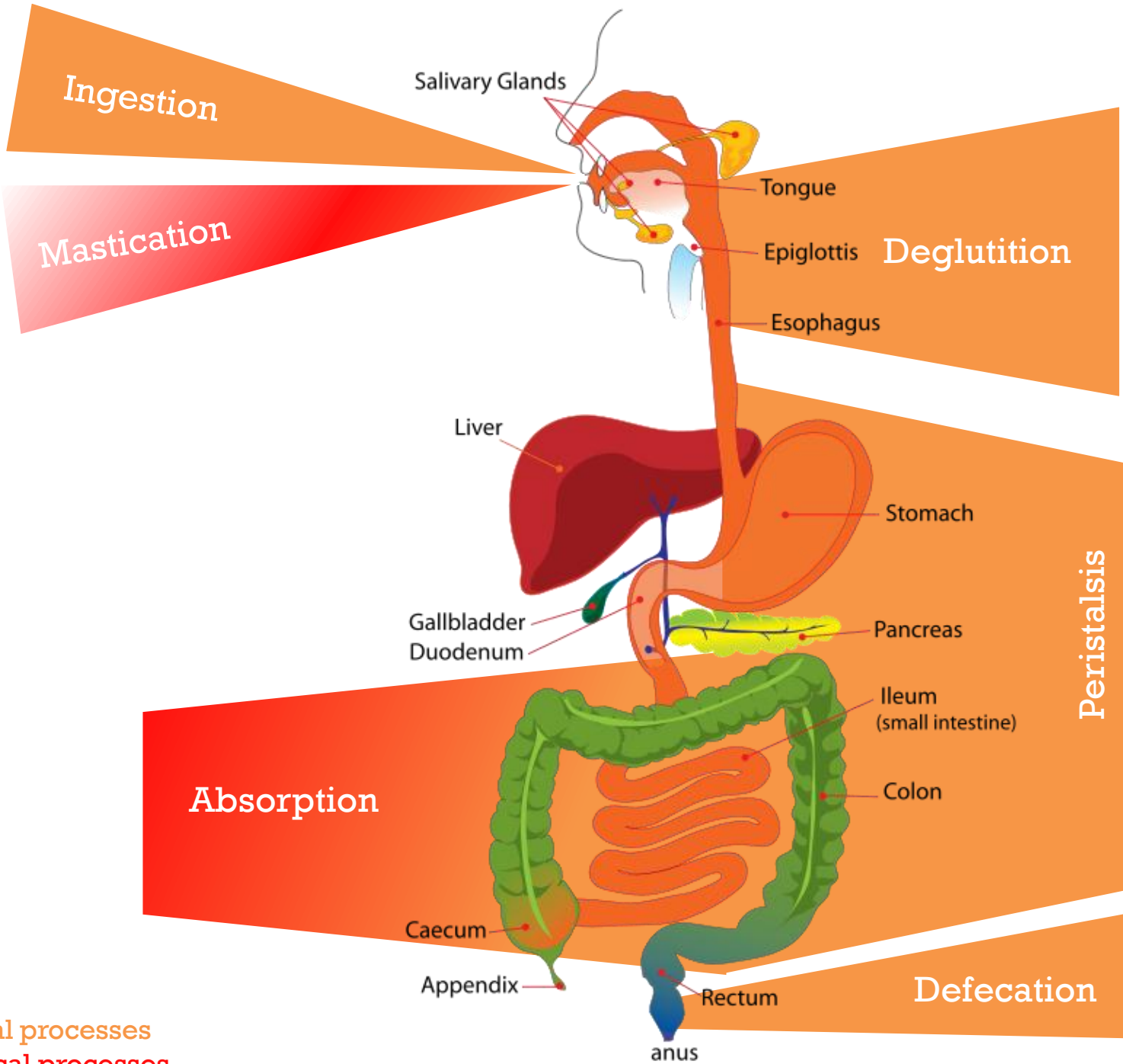


- **Gastrointestinal system**
- **Long tube**
- **Lumen open to external environment**



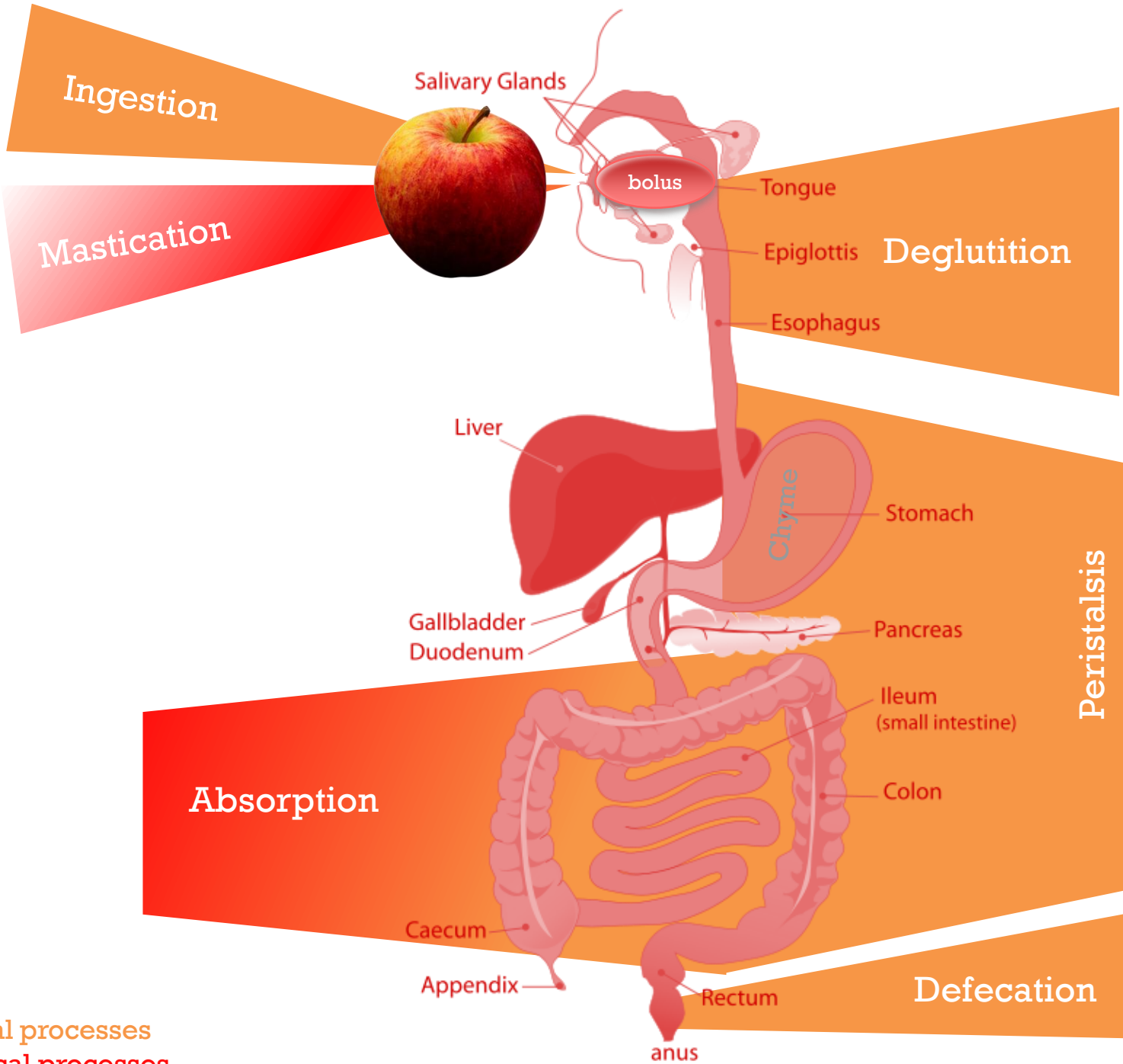
The Digestive System

The Digestive System



Physical processes
Chemical processes

The Digestive System



Physical processes
Chemical processes



Salivary Glands

Saliva → moisture, amylase

Tongue

• Pepsinogen, HCl and hormone production
• Alkaline mucus to protect lining
• Muscular contraction to mix contents

Epiglottis

Esophagus

Bile:
• Produced in liver
• Stored in gallbladder
• Fat emulsification

Liver

Stom

Pancreatic secretions:
• Bicarbonate
• Proteases
• Lipases
• Nucleases

Gallbladder
um

Breakdown & absorption
3 sections:
• Duodenum
• Jejunum
• Ileum
Produces:
• Mucus
• Enzymes
• Hormones

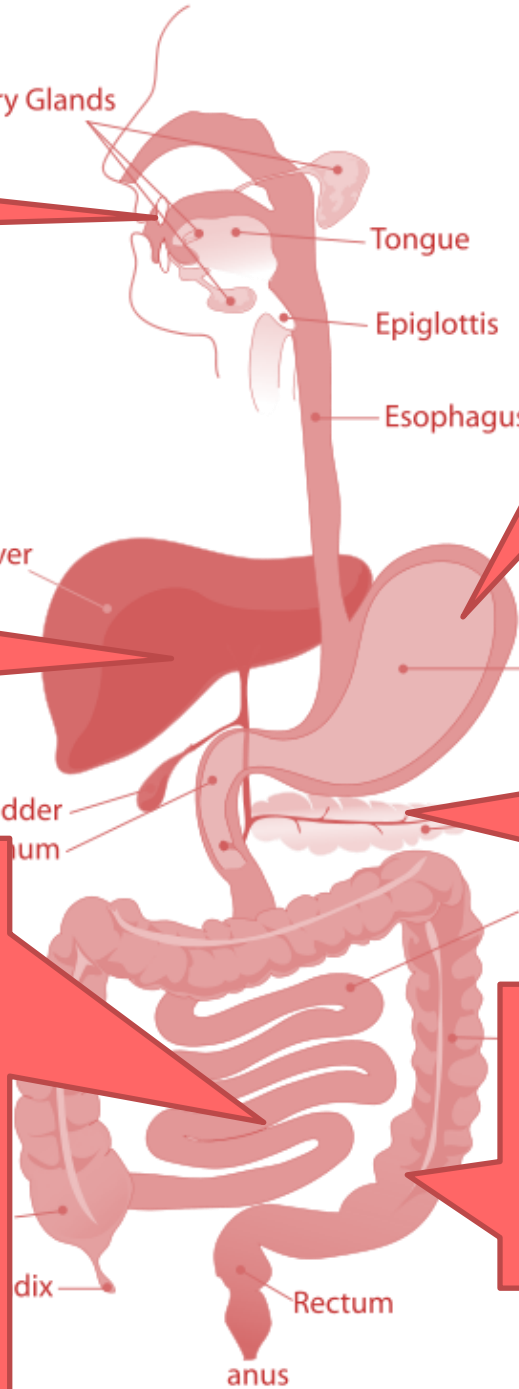
Ileum (small intestine)

4 sections:
• Ascending } Water
• Transverse } Sodium
• Descending }
• Sigmoid } Storage

dix

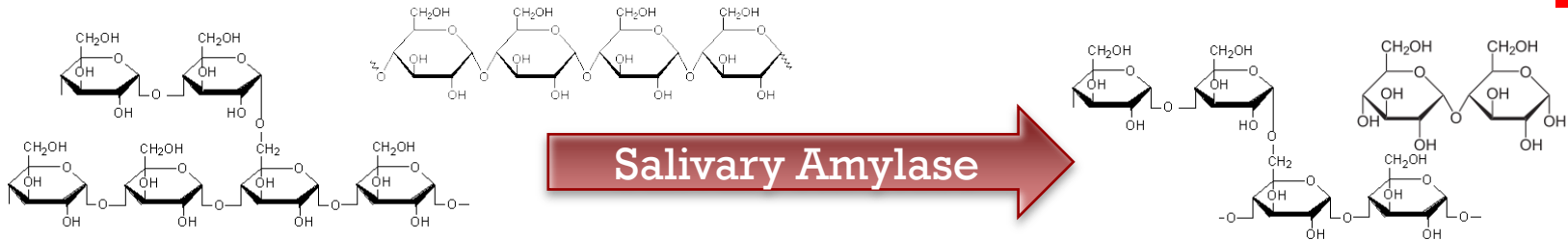
Rectum

anus

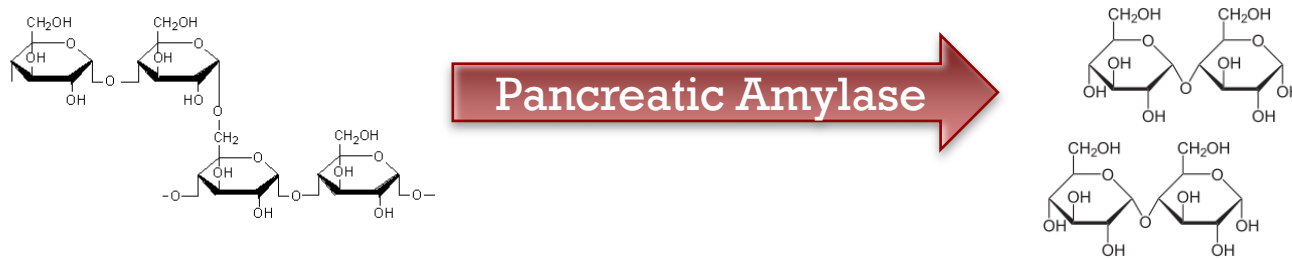


+ Carbohydrate Digestion

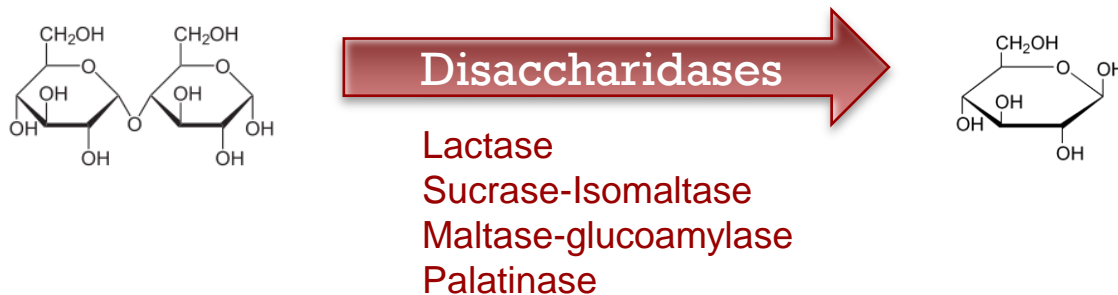
■ Digestion begins in mouth



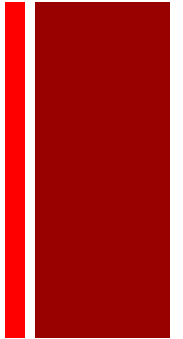
■ Continues in small intestine



■ And at the brush border



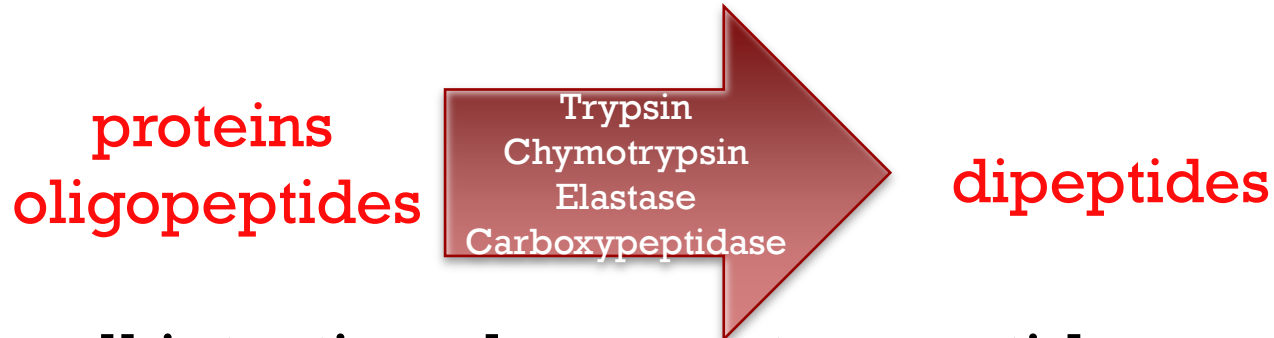
+ Protein Digestion



- Pepsinogen is converted to pepsin in the stomach



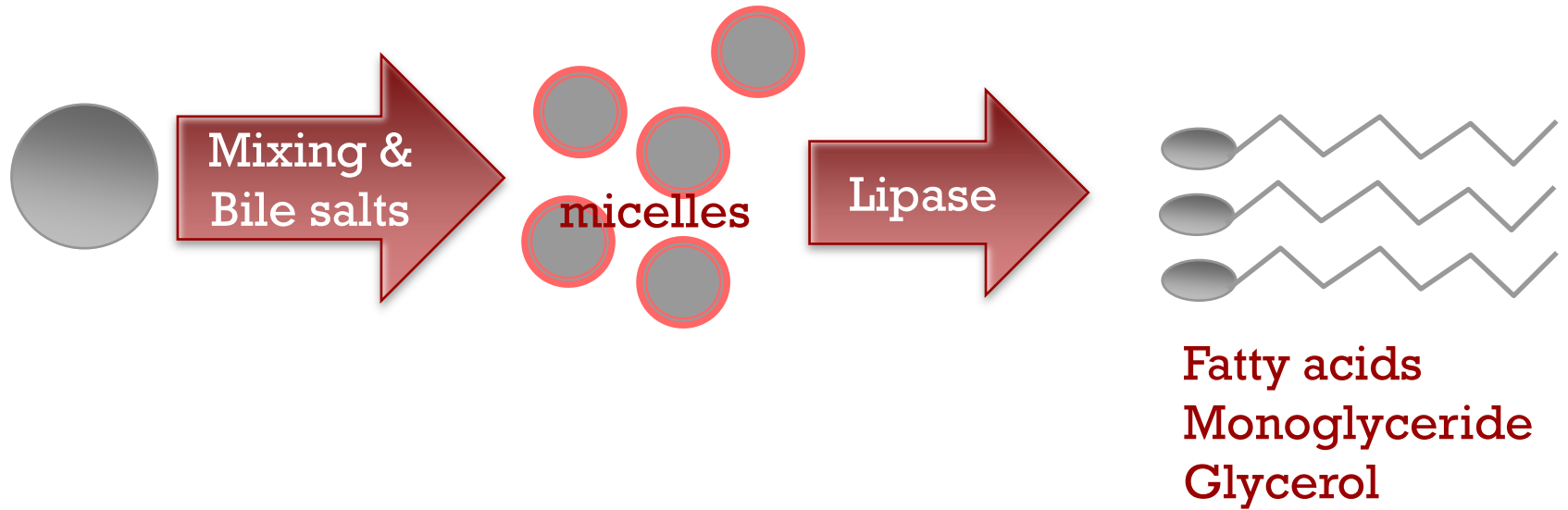
- Pancreatic proteases are activated in the small intestine



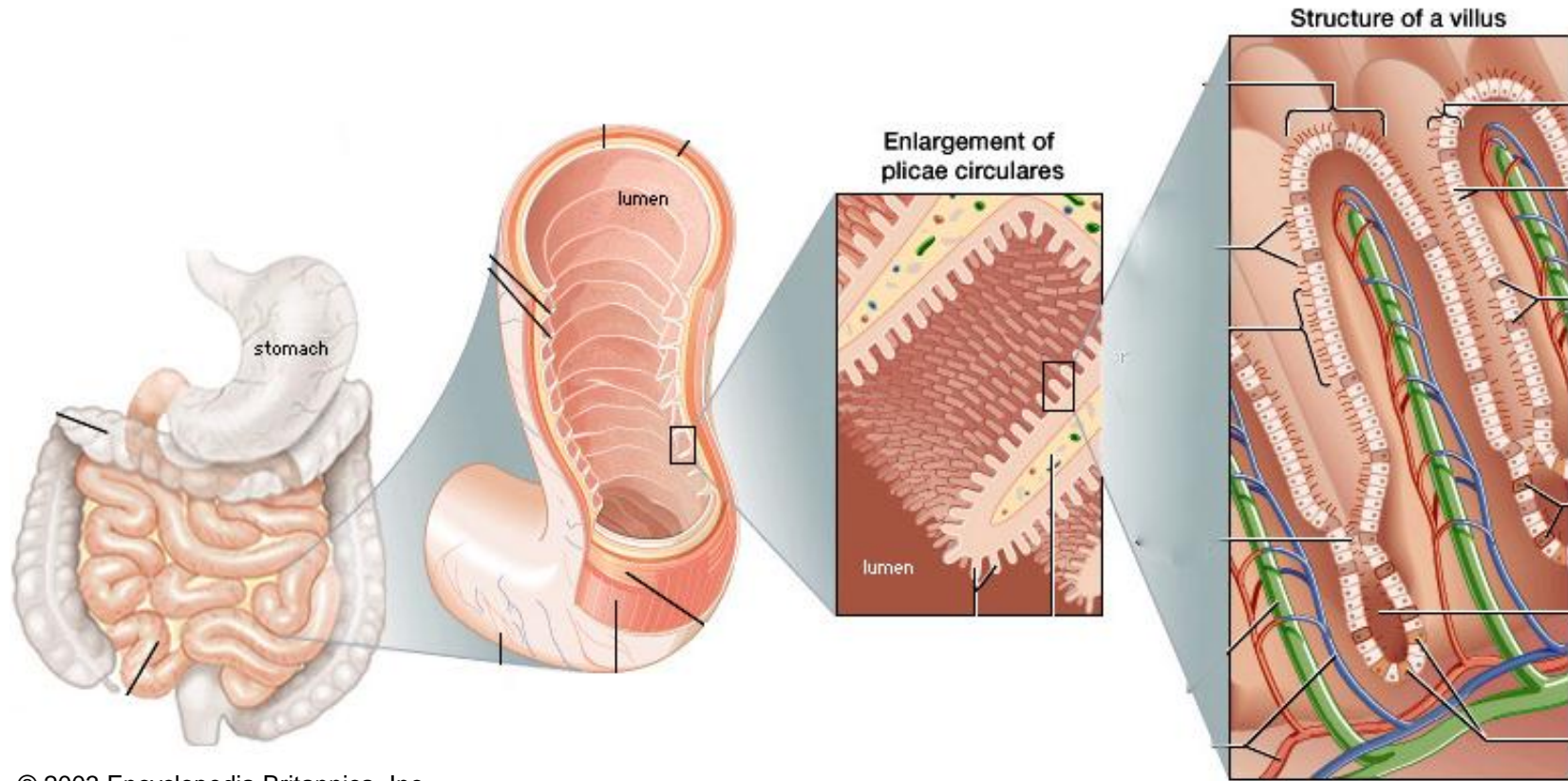
- Small intestine also secretes peptidases



+ Fat Digestion

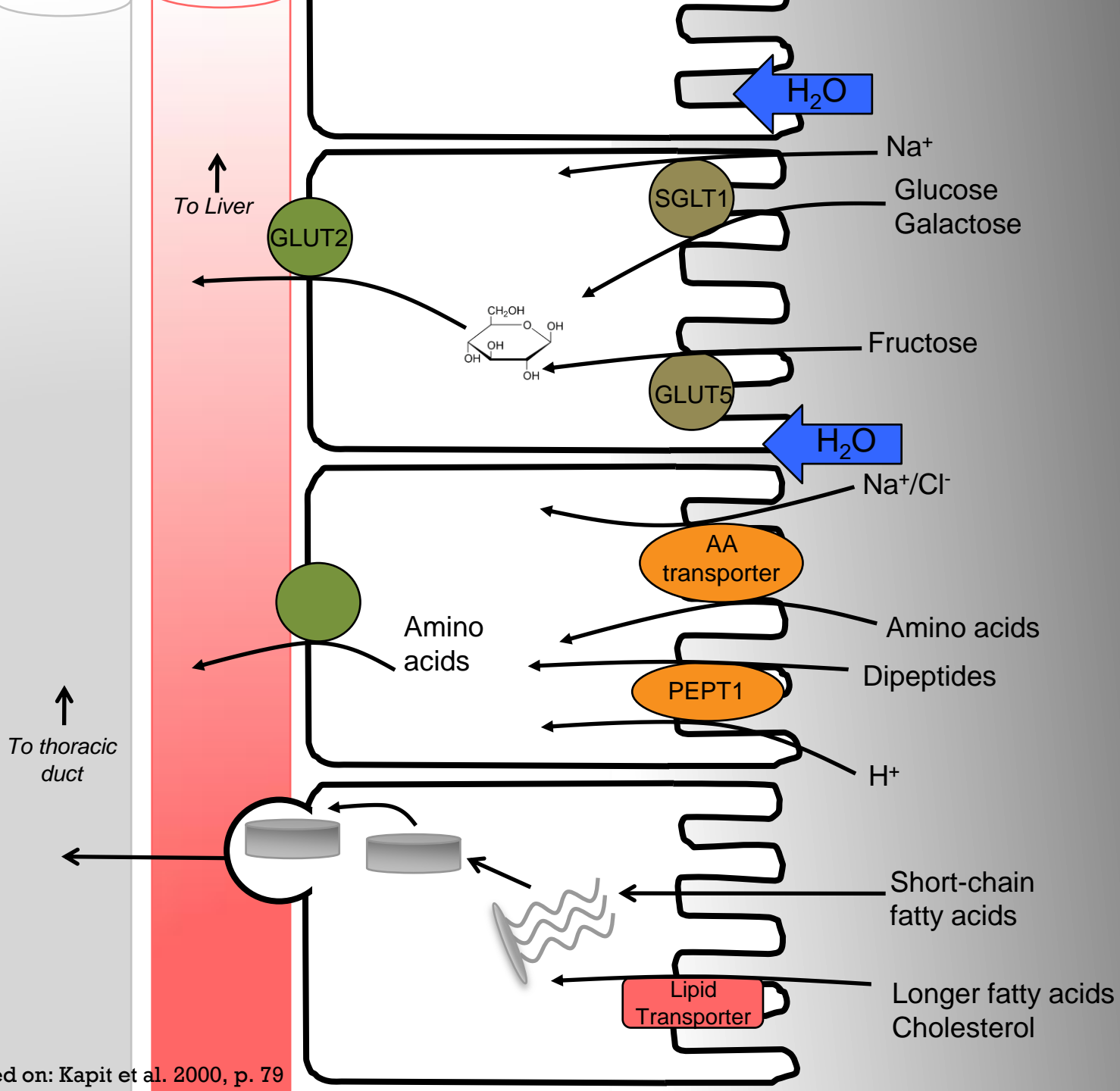


+ Nutrient Absorption

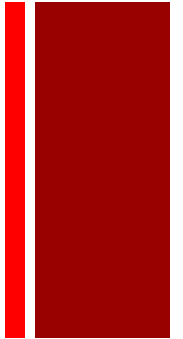


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Nutrient Absorption



+ Causes of Malabsorption



Digestion

Luminal

Absorption

Mucosal

+ Causes of Malabsorption

Impaired Digestion

- Abnormal physical processes
 - Inadequate chewing
- Inadequate digestive secretions
 - Bile acid disorders
 - Obstructions (bile or pancreatic duct)
 - Liver dysfunction
 - Bacterial overgrowth
- Enzyme insufficiency
 - Pancreatic insufficiency
 - Chronic pancreatitis
 - Cystic Fibrosis
 - Shwachman-Diamond Syndrome
 - Zollinger-Ellison Syndrome
 - Disaccharidase deficiency
 - Congenital/genetic deficiencies

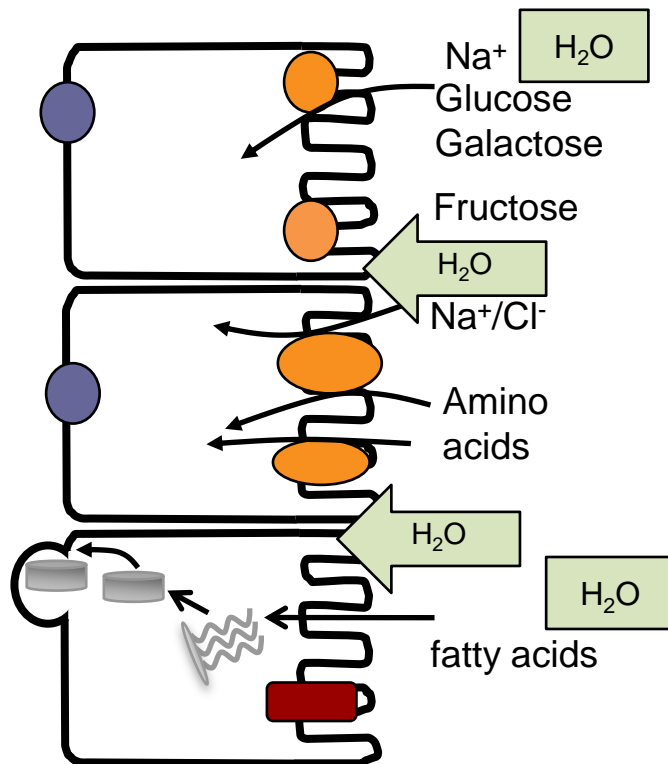
+ Causes of Malabsorption

Impaired Absorption

- Physical damage
 - Short bowel syndrome
 - Bowel obstructions
 - Intestinal tissue damage
 - Celiac disease
 - Crohn's disease
 - Whipple's disease
- Transporter/cofactor deficiencies
 - Hartnup's → amino acid transport
 - SGLT-1 → glucose/galactose transport
 - Intrinsic factor → Vitamin B12 transport

+ Symptoms of Malabsorption

■ Undigested/unabsorbed molecules in the GI tract



- Osmotic diarrhea
- Nutrients reach colon
 - Excreted in feces
 - Steatorrhea
 - Protein
 - Sugars
- Bacterial fermentation
 - Flatulence
 - Acid production



+ Symptoms of Malabsorption

- Undigested/unabsorbed active molecules in the GI tract
 - Diarrhea
 - Flatulence
 - Bloating
- Malnutrition, failure to thrive
 - Nutrient insufficiency
 - Weight loss
 - Wasting
 - Fatigue
 - Anemia

Symptoms are non-specific

+ An unsettled stomach

- 9 month old girl
- Skin rash → antibiotics
- Diarrhea next day
- Switch to soy-based formula, then protein hydrolysate
- Hospitalized
 - Staphylococcal scalded skin syndrome
 - Negative: toxins, ova, parasites, bacteria
 - Normal: CBC, electrolytes

Rule-out other causes of symptoms:
Imaging
Inflammatory markers
Bleeding/organ damage
Infection

+ Laboratory Evaluation of Malabsorption

- Look for unabsorbed nutrients in feces and urine
 - Sugars, fat, protein
- Look for absence of nutrients in circulation
 - Oral glucose load
- Look for indirect evidence of malabsorption
 - Stool pH
 - Hydrogen breath test
 - Fat-soluble vitamin deficiencies
- Look for the presence and activity of digestive enzymes
 - Disaccharidase activity
 - Fecal elastase, trypsin
 - Enzyme levels in duodenal aspirates
- Look for evidence of GI damage
 - Xylose absorption test
 - Endomesial and gliadin antibodies
 - Inflammatory markers

+ An unsettled stomach

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- Hospitalized

Staphylococcal scalded skin syndrome

Negative: toxins, ova, parasites, bacteria

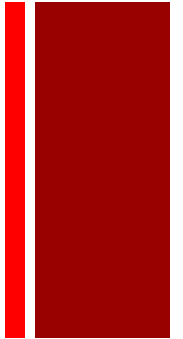
Normal: CBC, electrolytes

Stool analysis: 0.75-2% reducing substances
pH 5

- Diarrhea remitted when oral feedings were stopped;
resumed when oral feedings were resumed



+ Laboratory Evaluation of Malabsorption

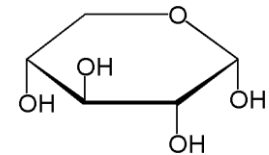


- Look for unabsorbed nutrients in feces and urine
- Look for absence of nutrients in circulation
- Look for indirect evidence of malabsorption
- Look for the presence and activity of digestive enzymes
- **Look for evidence of GI damage**

+ D-xylose absorption

Mucosal permeability of small intestine

- Pentose monosaccharide passively absorbed in proximal small bowel
- Excreted in urine



Procedure:

Overnight fast
5 or 25 g oral dose of D-xylose
5 hour urine collection
(1 or 2 hour blood collection)

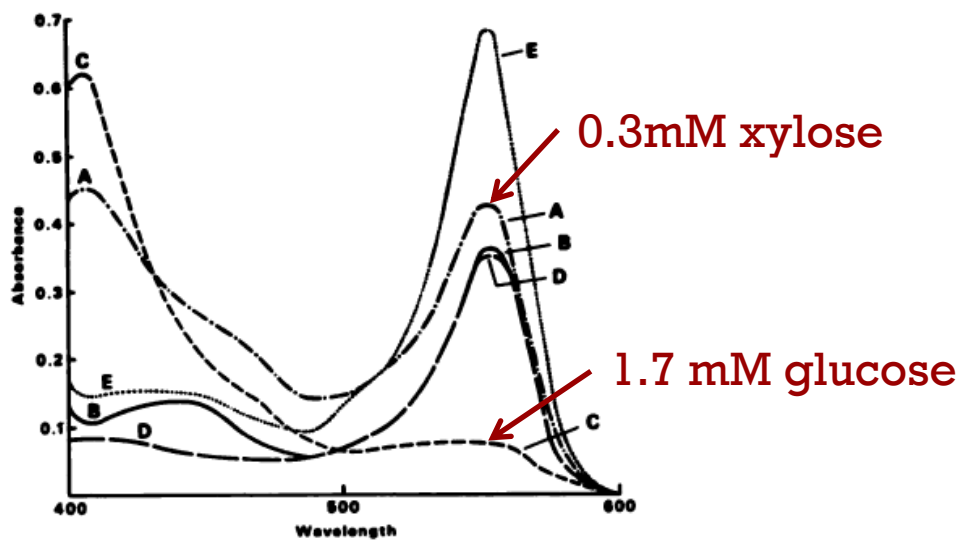
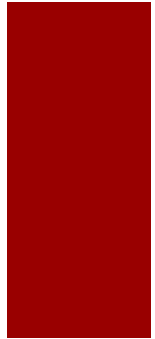
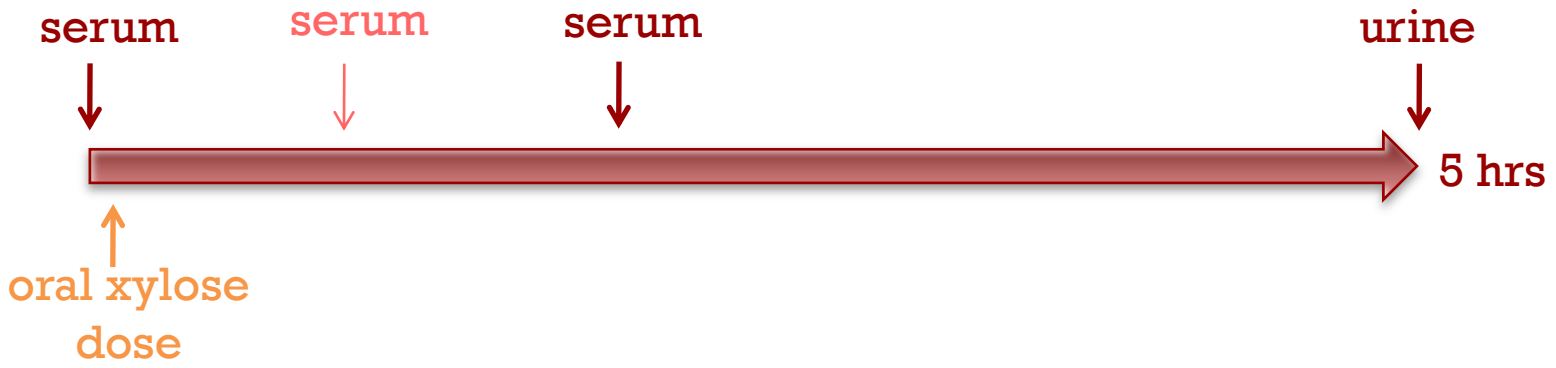
xylose + phloroglucinol

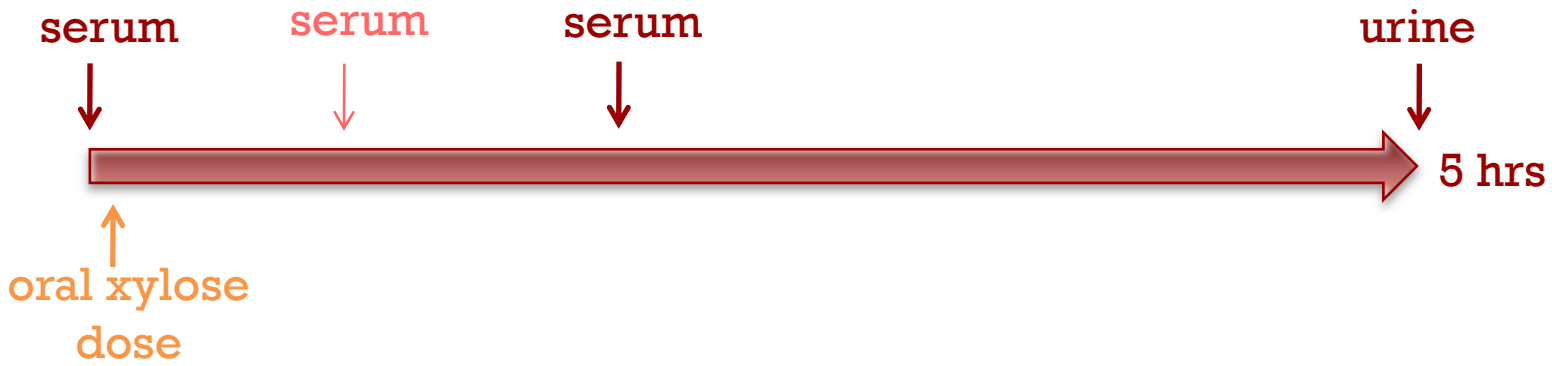


acid
heat

product (Abs 540nm)

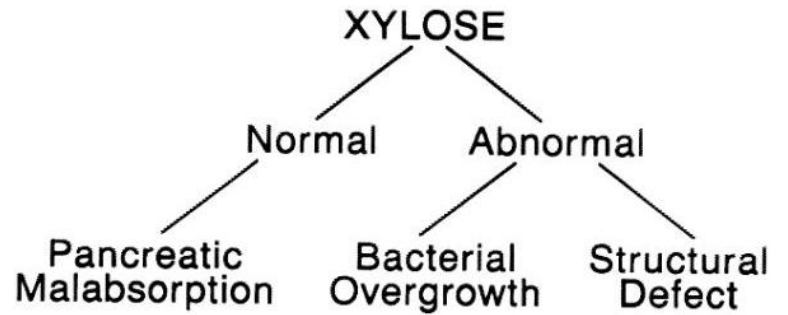
+





Reference Intervals (adults, 25 g dose)

2 hr serum	32-58 mg/dL
5 hr urine – fraction	14-40%
– amount	3.5-10 g/hour



+ Laboratory Evaluation of Malabsorption

- Look for unabsorbed nutrients in feces and urine
- Look for absence of nutrients in circulation
- Look for indirect evidence of malabsorption
- Look for the presence and activity of digestive enzymes
- Look for evidence of GI damage





Fecal Fat Testing

Evidence of unabsorbed nutrients

- Steatorrhea → pancreas, bile acid, damage, transport, mixing diagnosing fat malabsorption
- "Gold standard" for diagnosis
- Treatment monitoring
- Method history: solvent extractions, titrimetric or gravimetric, FTIR

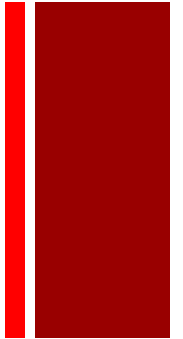
Procedure:

3 day stool collection
Normal (50-150 g/day) fat diet
No barium, charcoal or non-digestible fat intake
Sample is weighed and dried

Method:

NMR
Calibrated
Quantitation of % fat
Calculated weight/day result

+ H^1 NMR



- Nuclear magnetic resonance
- Proton in a magnetic field excited by radio-frequency pulse resonates at a particular frequency which then decays over a period of time
- Signal decay is slower when protons are in lipids than in other substances
 - Signal isolation
 - % Fat determined using calibration curve

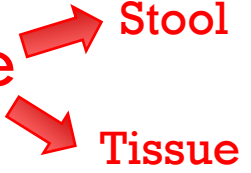
Quantitative Fecal Fat

Reference Interval (72 hr collection)

0-5 years	0 - 2.0 g/24h
≥ 6 years	0 - 6.0 g/24h

+ Laboratory Evaluation of Malabsorption



- Look for unabsorbed nutrients in feces and urine
- Look for absence of nutrients in circulation
- Look for indirect evidence of malabsorption
- Look for the presence and activity of digestive enzymes A diagram consisting of two red arrows originating from the word 'digestive' in the fourth bullet point. One arrow points diagonally upwards and to the right towards the word 'Stool'. The other arrow points diagonally downwards and to the right towards the word 'Tissue'.
- Look for evidence of GI damage

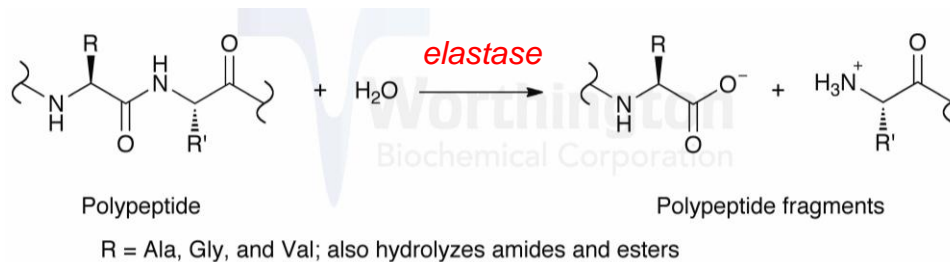
+ Fecal Elastase

Pancreatic exocrine function, protease enzymes

- Produced by pancreas



- Serine protease, hydrolyzes amide and ester bonds



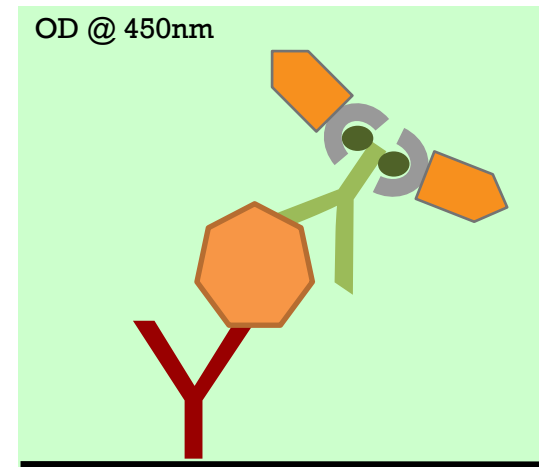
- Remains intact and active in the intestine
- Concentrated in feces versus duodenal fluid

+ Fecal Elastase

- Enzyme-linked immunoassay
- Stool homogenates
- Double-sandwich, signal amplification
- Species and tissue-specific antibodies

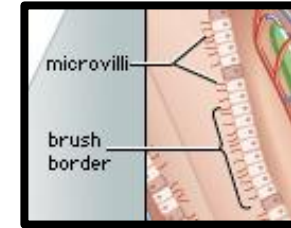


Pancreatic Elastase ($\mu\text{g/g}$ feces)	
Normal	201-500
Mild-moderate insufficiency	100-200
Severe insufficiency	≤ 99

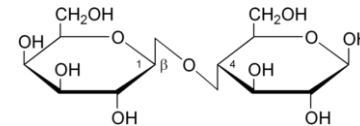


+ Disaccharidase Assay

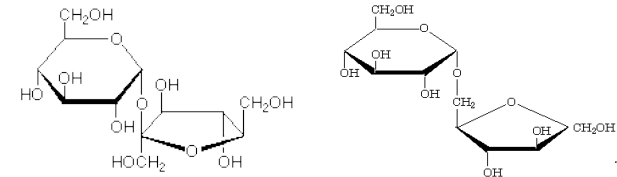
Brush-border disaccharidase activity



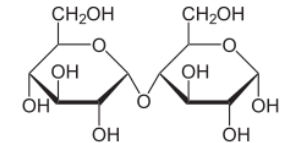
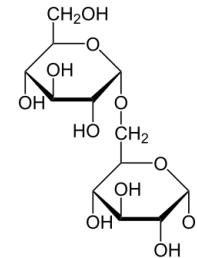
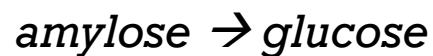
- Lactase



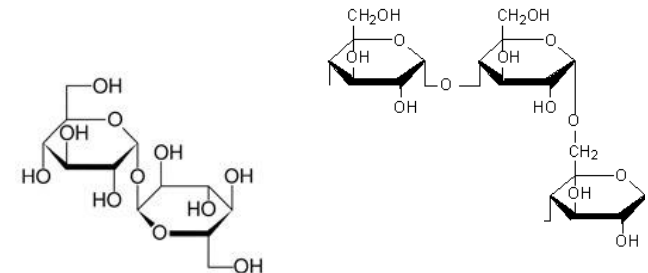
- Sucrase-Isomaltase



- Maltase-Glucoamylase



- Trehalase



* a.k.a isomaltulose

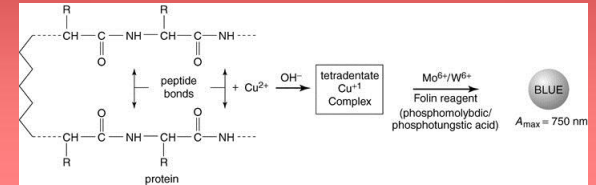
+ Dissacharidase Assay

■ **Sample:** ~ 5 mg intestinal biopsy

■ **Procedure:** Homogenize tissue



Assay total protein
Lowry-based method



Test individual dissach activity

lactase
Lactose → *glucose + galactose*

β-I
Sucrose → *glucose + fructose*

S-I
Palatinose → *glucose + fructose*

S-I / M-G
Maltose → *glucose + glucose*

Saline → *blank/endogenous sugar*

60', 37°C



30', 37°C

Cobas Glucose Assay

Glucose + ATP → *G-6-P* + ADP

G-6-P + NADP⁺ → *gluconate-6-P* + NADPH + H⁺
340 nm





For each sample:

Total protein

Glucose derived from lactose (Lactase, β -1,4-activity)

Glucose derived from sucrose (S-I, α -1,2-activity)

Glucose derived from palatinose (S-I, α -1,6-activity)

Glucose derived from maltose (S-I, α -1,4-activity)

Baseline glucose in sample (blank)



Enzyme activity (rate of glucose formation) normalized to total protein



$\mu\text{mol}/\text{min}/\text{g}$ total protein

Reference Intervals ($\mu\text{mol}/\text{min}/\text{g}$)

Lactase	≥ 15
Maltase	≥ 100
Sucrase	≥ 25
Palatinase	≥ 5

- Primary disaccharidase deficiency can be established only in the absence of intestinal injury
- Lactase deficiency
 - Age-dependent onset
 - 80-100% prevalence in some groups
- Sucrase-isomaltase deficiency
 - Congenital, gene mutations affect processing
 - Rare, 0.2-5% prevalence



+ An unsettled stomach

- 9 month old girl
- Skin rash → antibiotics
- Diarrhea next day
- Switch to soy-based formula, then protein hydrolysate

- Hospitalized

Staphylococcal scalded skin syndrome

Negative: toxins, ova, parasites, bacteria

Normal: CBC, electrolytes

Stool analysis: 0.75-2% reducing substances

pH 5

- Diarrhea remitted when oral feedings were stopped; resumed when oral feedings were resumed
- Disaccharidase testing showed low sucrase-isomaltase activity

Disaccharidase Activity ($\mu\text{mol}/\text{min}/\text{g}$)		Reference Interval
Lactase	33.4	≥ 15
Maltase	20.3	≥ 100
Sucrase	0.6	≥ 25
Palatinase	3.2	≥ 5

+ Management of Malabsorption

- Treat underlying conditions
 - Antibiotics, surgery, anti-inflammatory agents
- Nutritional supplementation
 - Vitamins and minerals
 - Parenteral nutrition
- Avoid sources of offending substances
 - Disaccharide-containing foods (milk, table sugar)
 - Gluten-free diet

- Enzyme Replacement
 - Disaccharidases
 - Pancreatic enzymes



- Pre-treated/modified foods
 - Yogurt & aged cheese (bacterial fermentation)
 - Lactose-reduced milk
 - Shorter-chain fats → less dependent on bile and lymphatics

+ An unsettled stomach

- 9 month old girl
- Skin rash → antibiotics
- Diarrhea next day*
- Switch to soy-based formula, then protein hydrolysate**
- Cow's milk formula did not produce symptoms
- Avoiding sucrose or isomaltose-containing formula prevented symptoms



* The antibiotic preparation contained sucrose

** Alternative formula preparations contained corn syrup solids



Conclusions

Thanks! Any questions?

- Causes of malabsorption may arise from disruption of physical and/or chemical processes of digestion as well as impairments in nutrient absorption
- Laboratory methods employing a wide variety of methodologies can help in the evaluation of suspected malabsorption and help to identify the underlying causes
- Patient management will depend on the underlying cause and can include dietary modification as well as supplementation

Special thanks to the Sp Chem and PAFT labs.



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