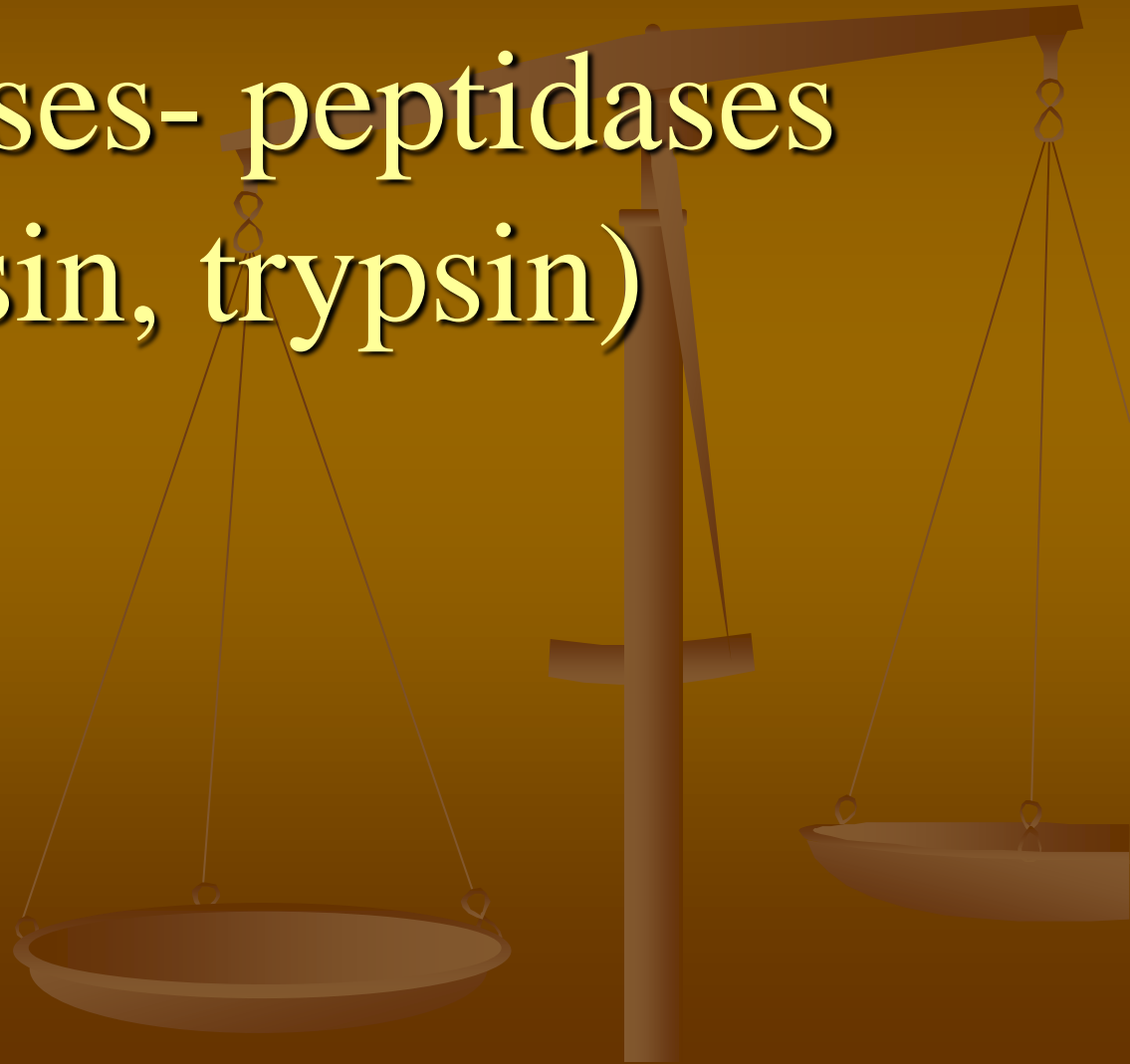
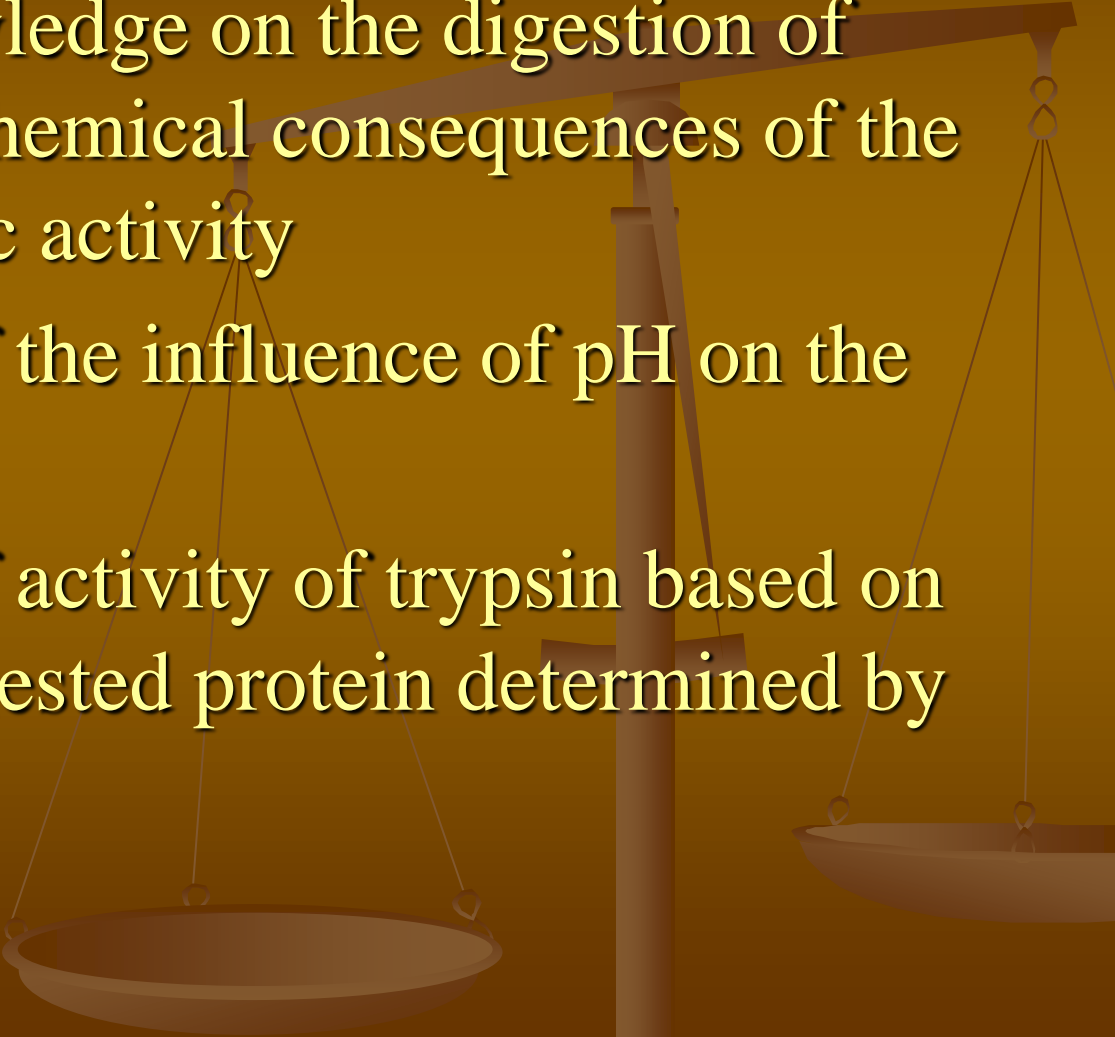


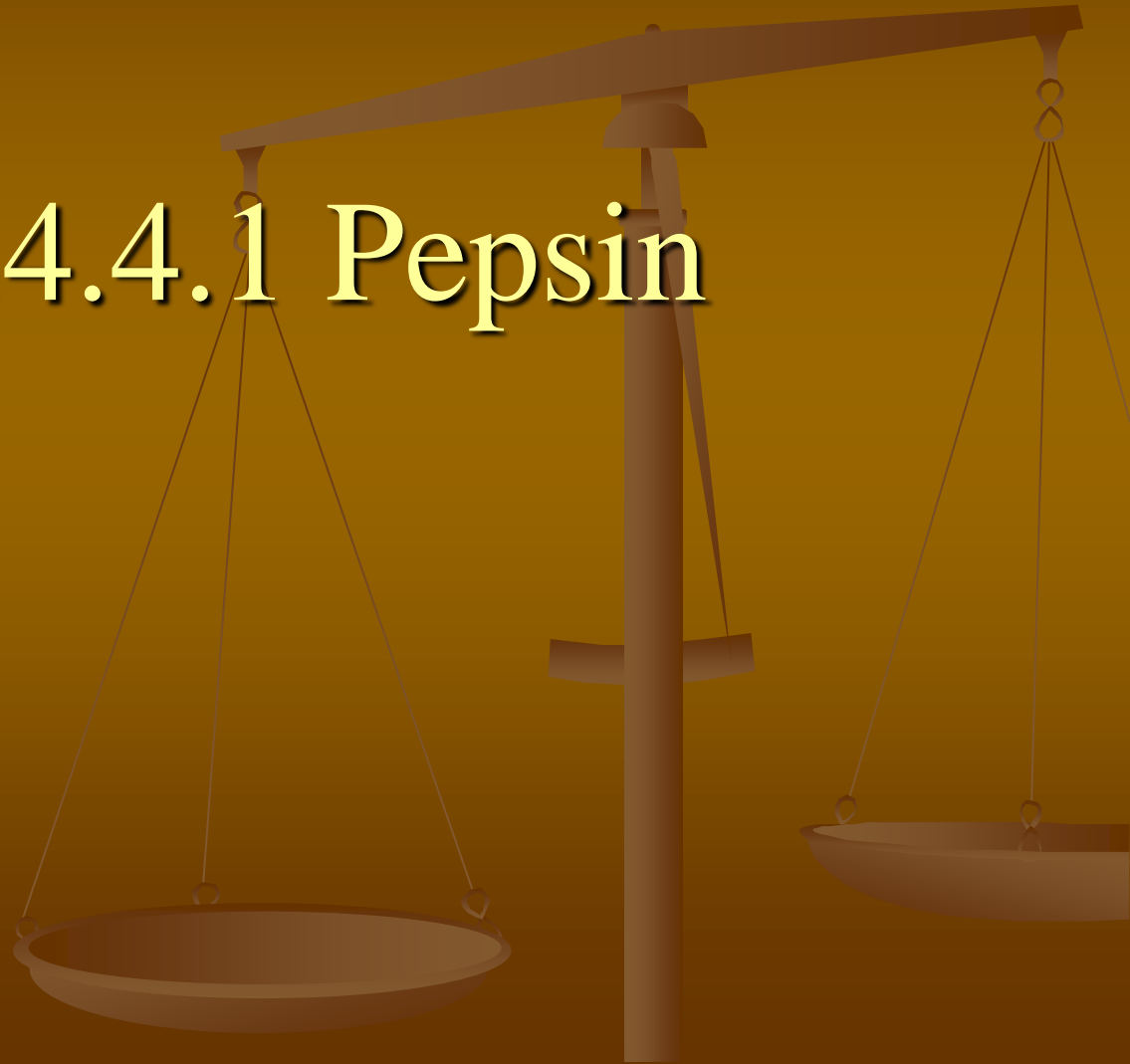
Hydrolases- peptidases (pepsin, trypsin)



The aim of classes:

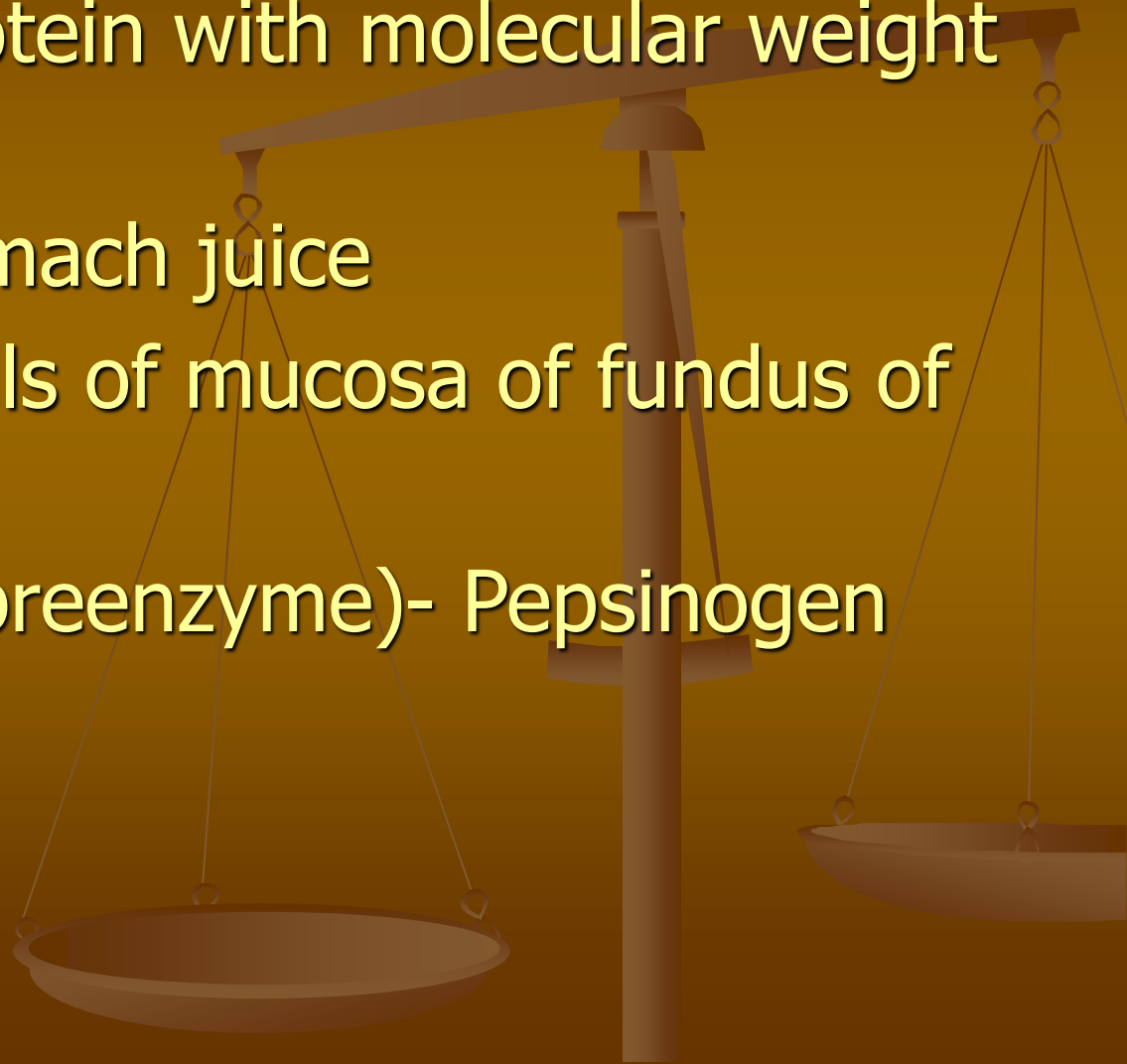
- Gaining the knowledge on the digestion of proteins and biochemical consequences of the lack of proteolytic activity
 - The estimation of the influence of pH on the activity of pepsin
 - The estimation of activity of trypsin based on the amount of digested protein determined by biuret method
- 

E.C.3.4.4.1 Pepsin



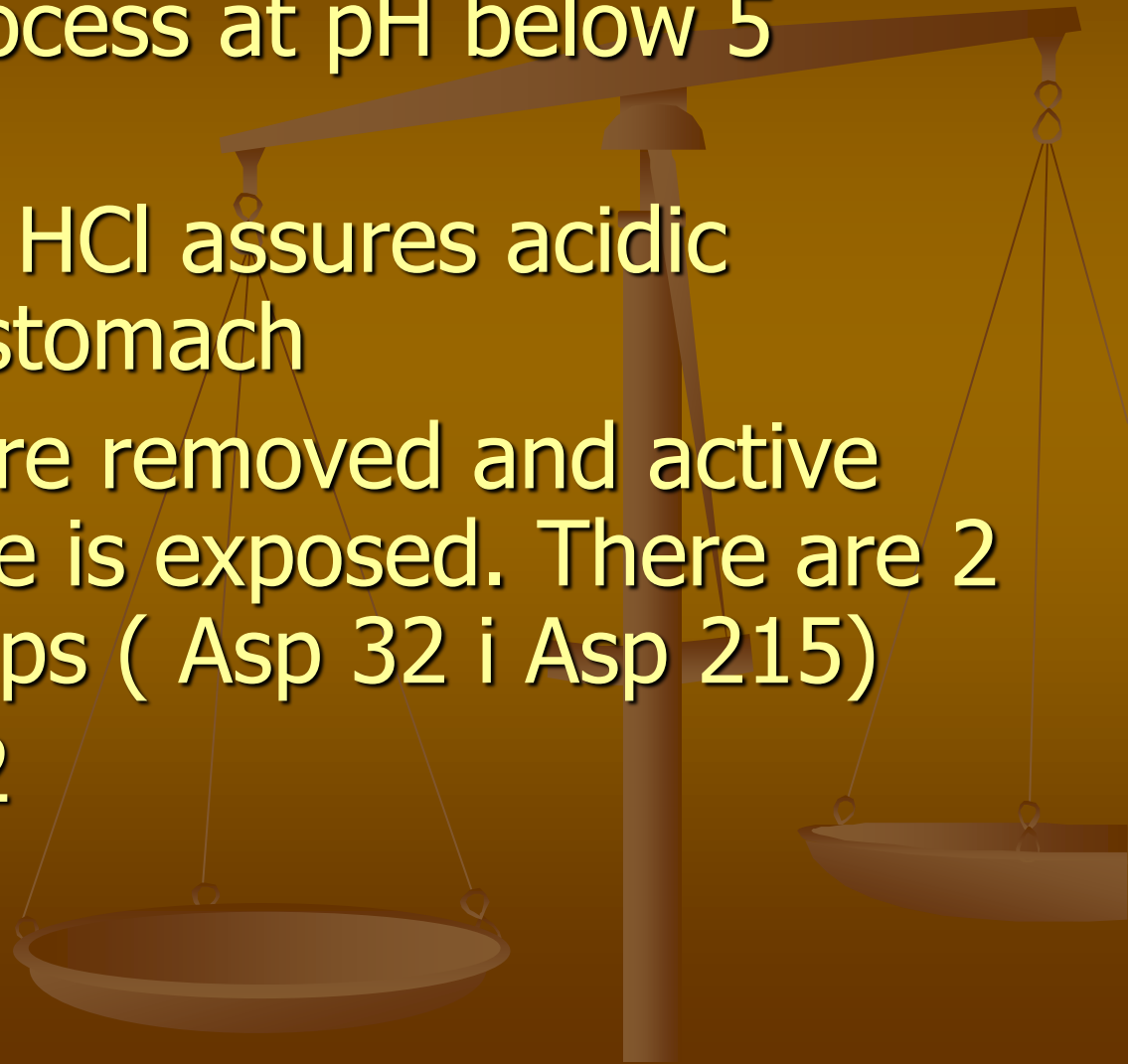
Characteristics:

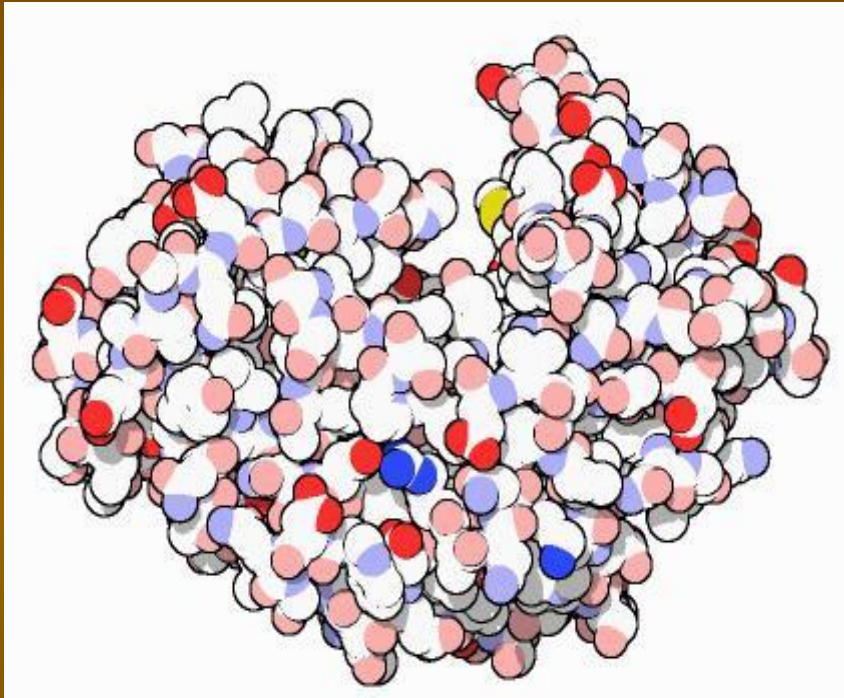
- Single chain protein with molecular weight of 34,5 kDa
- Protease of stomach juice
- Produced by cells of mucosa of fundus of stomach
- Inactive form (preenzyme)- Pepsinogen (43 kDa)



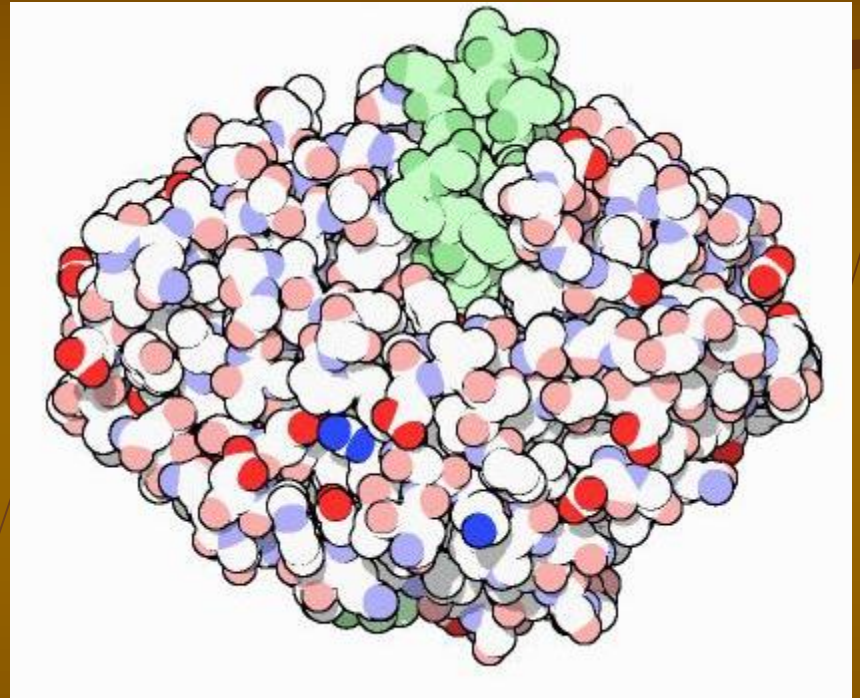
Activation:

- Spontaneous process at pH below 5 requires 3 steps
- The presence of HCl assures acidic environment in stomach
- 44 amino acids are removed and active center of enzyme is exposed. There are 2 β - carboxyl groups (Asp 32 i Asp 215)
- Optimum pH 1-2





PEPSIN



PEPSINOGEN

Action:

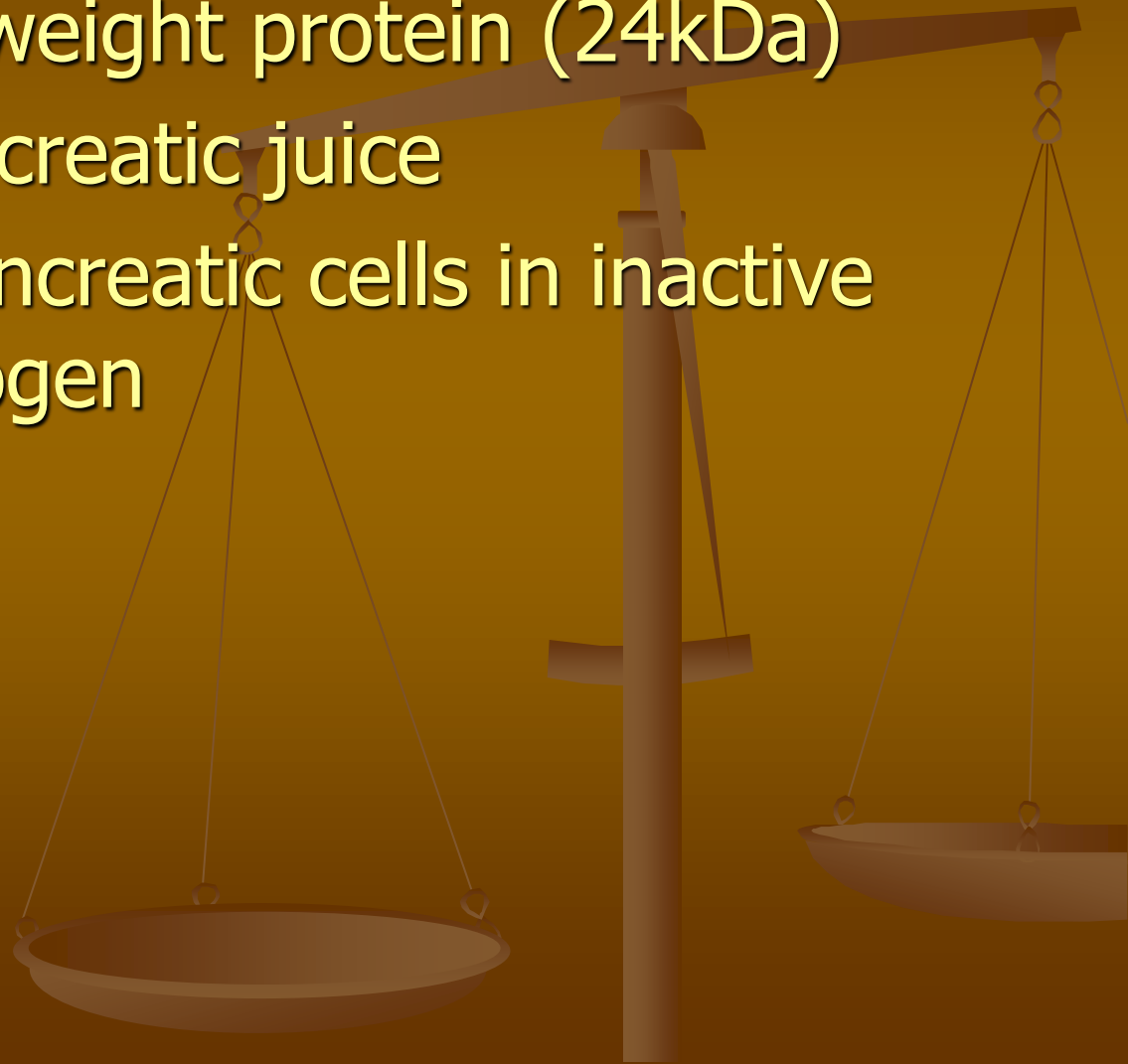
- peptidyl-peptide hydrolase which cleaves peptide bonds in proteins except from keratin and protamin
- Prefers bonds between phenyloalanin and tyrosin with other aminoacids as well as leucin and glutamic acid

E.C.3.4.4.4. Trypsin



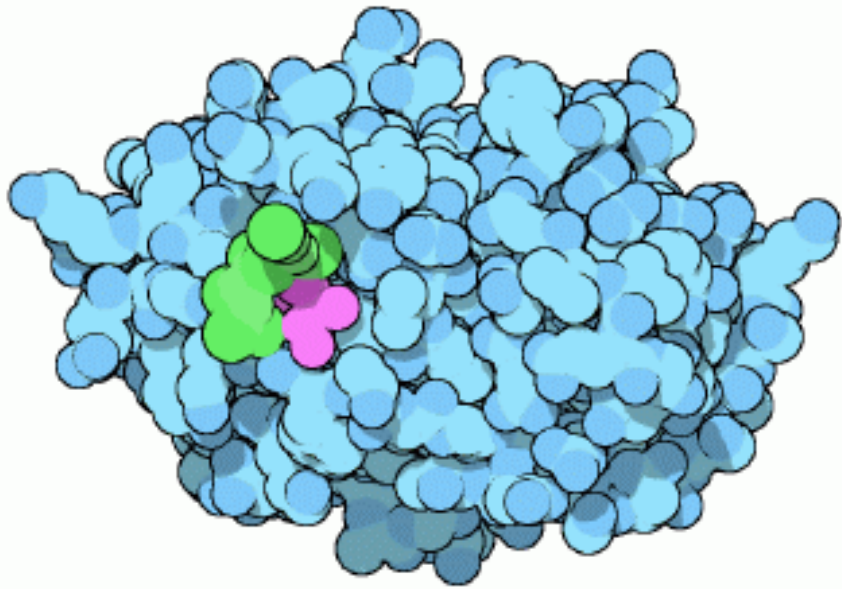
Characteristics:

- Low molecular weight protein (24kDa)
- Protease of pancreatic juice
- Produced by pancreatic cells in inactive form of trypsinogen

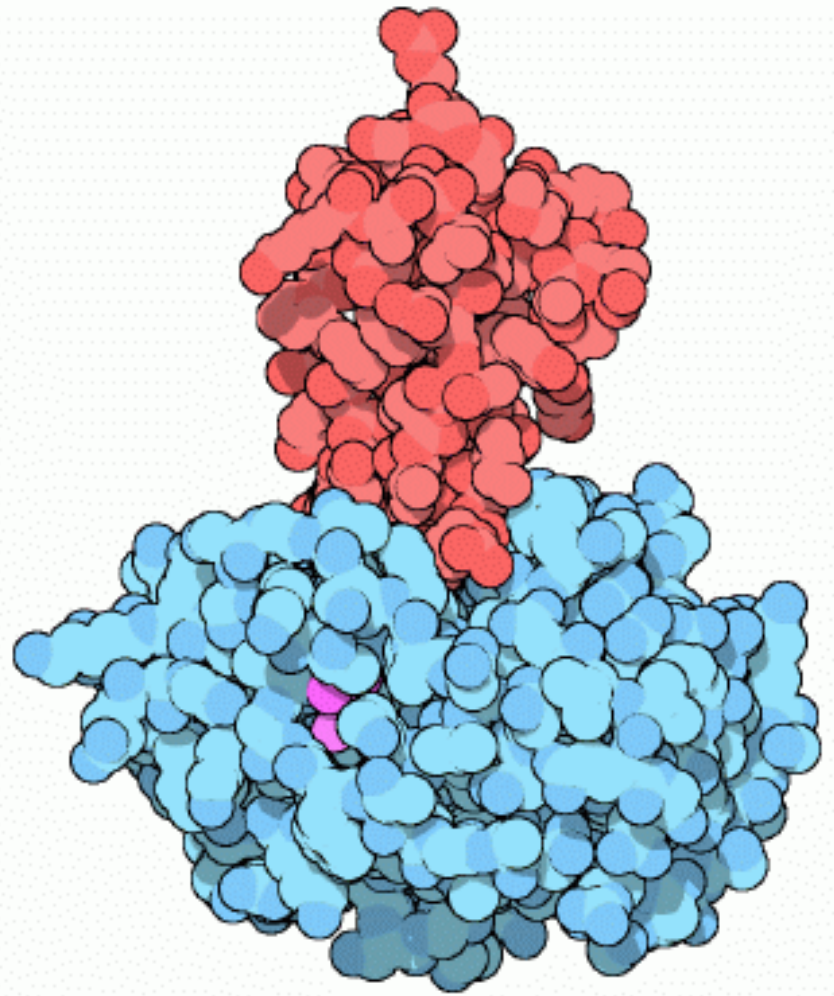


Activation:

- The participation of enterokinase (enzyme produced by duodenum) is indispensable for the activation
- It is autocatalytic process
- 6-aminoacid fragment is removed and active center of enzyme is exposed – main aminoacids are serine and histidine
- Active form of trypsin influences remaining zymogens of pancreatic juice and converts them into active forms eg. (chymotrypsinogen → chymotrypsin, proelastase → elastase, procarboxypeptidase → carboxypeptidase)
- Optimum pH 7-9



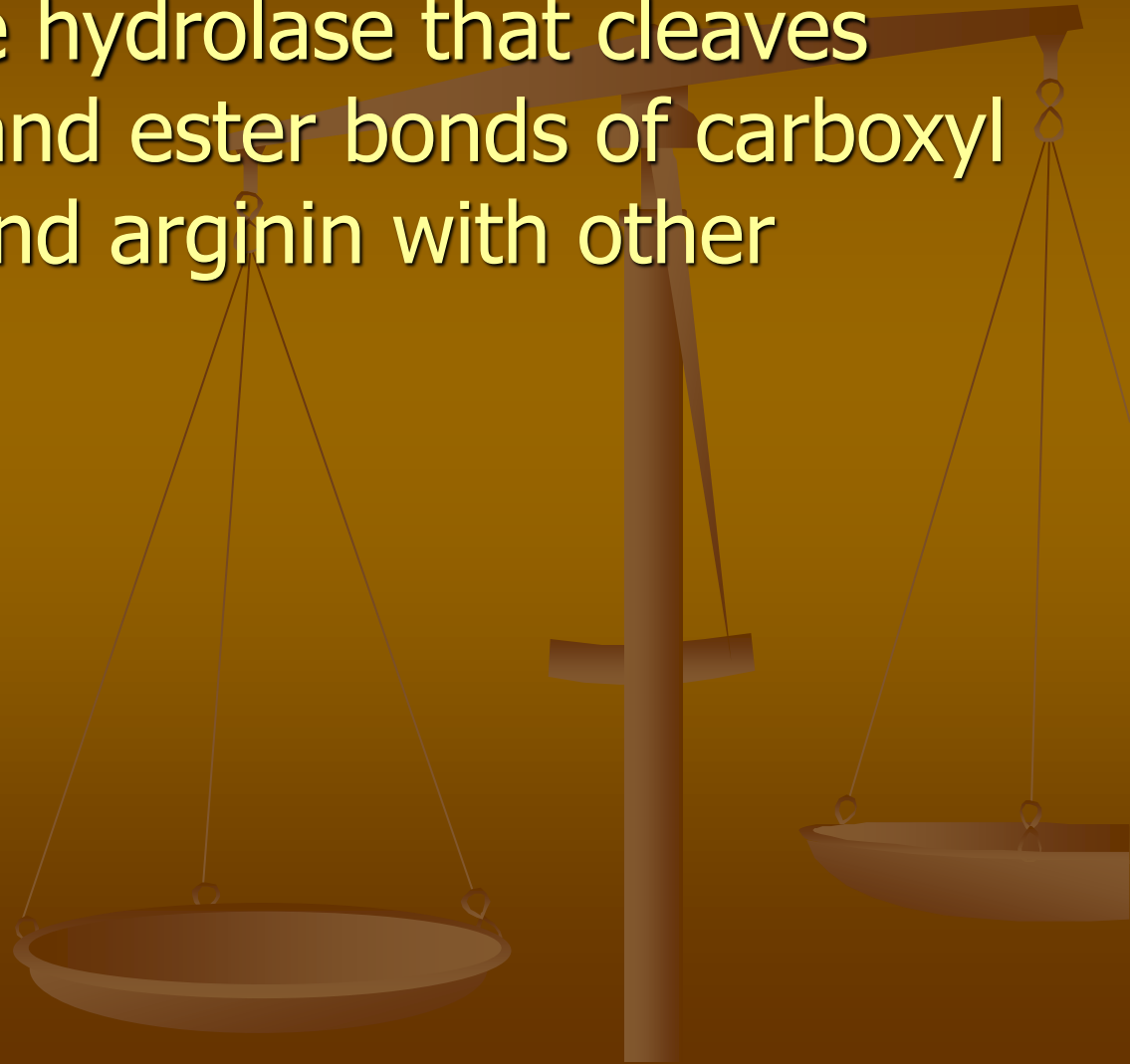
TRYPSIN



TRYPSINOGEN

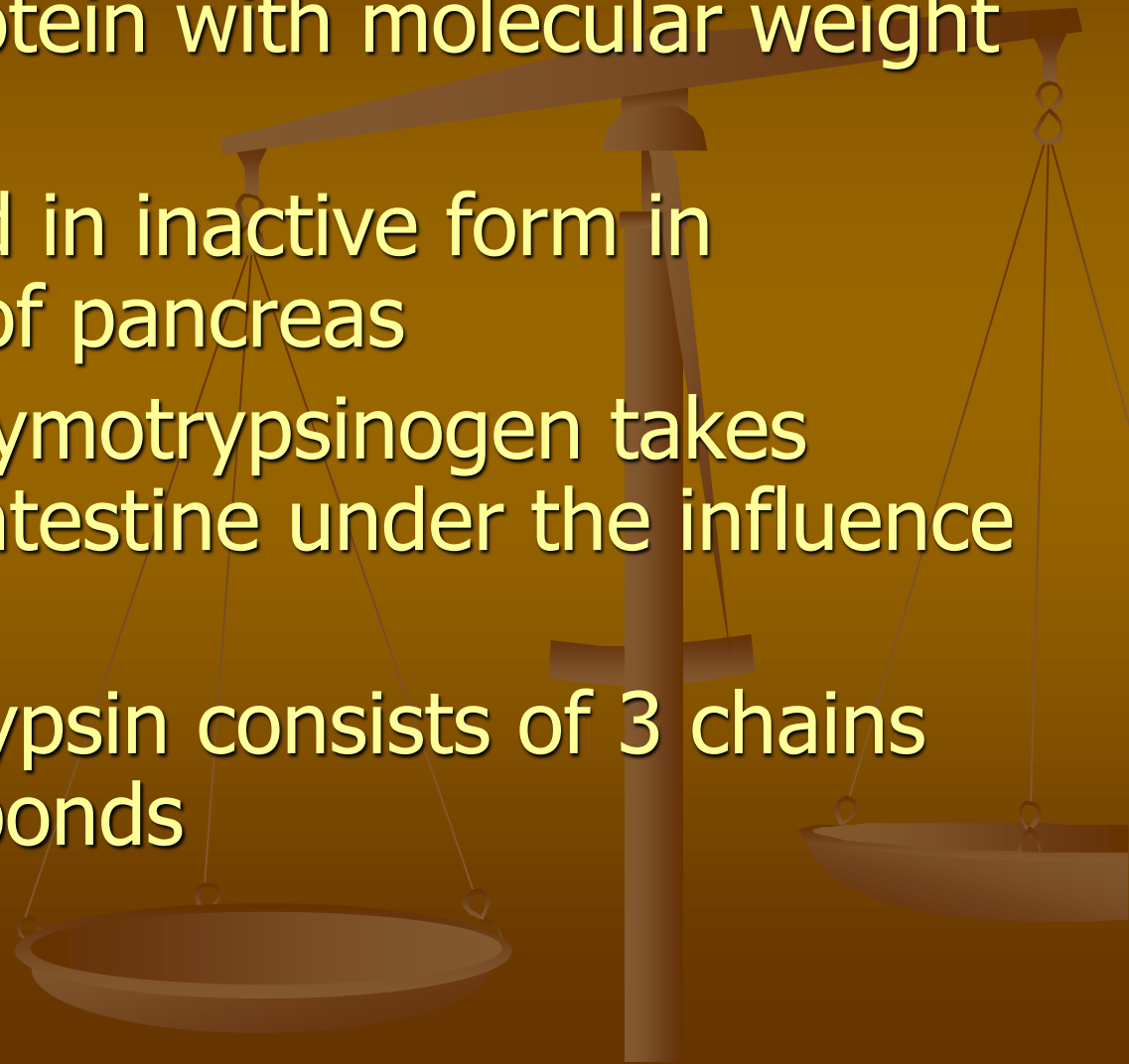
Action:

- Peptidyl-peptide hydrolase that cleaves peptide bonds and ester bonds of carboxyl group of lysin and arginin with other aminoacids



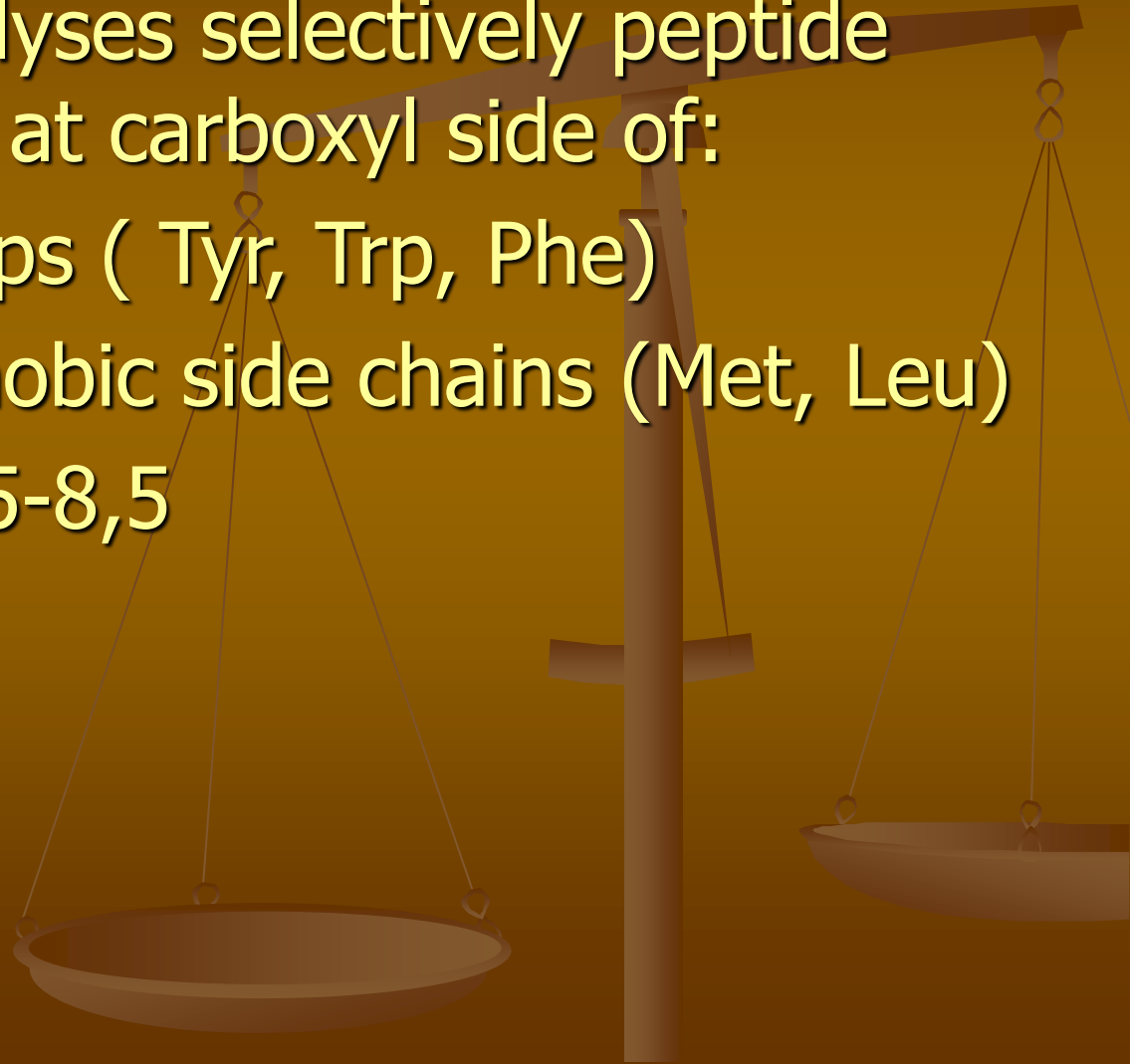
E.C.3.4.4.4. Chymotrypsin

- Single chain protein with molecular weight of 24 kDa
- It is synthesised in inactive form in glandular cells of pancreas
- Activation of chymotrypsinogen takes place in small intestine under the influence of trypsin
- Active Chymotrypsin consists of 3 chains linked by thiol bonds



Action

- Enzyme hydrolyses selectively peptide bonds located at carboxyl side of:
 - Aromatic groups (Tyr, Trp, Phe)
 - Large hydrophobic side chains (Met, Leu)
 - Optimal pH 7,5-8,5



Biochemical consequences:

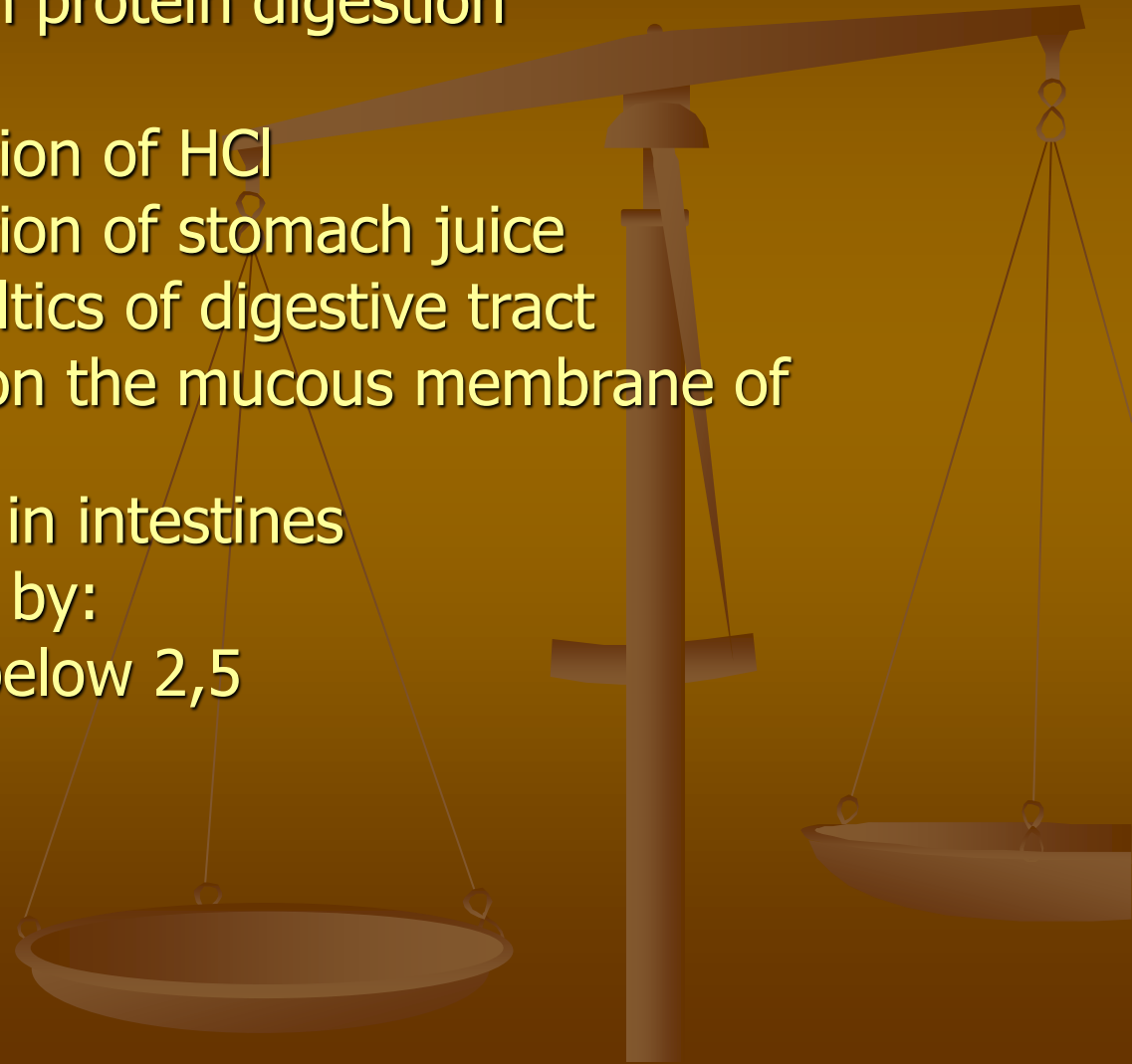
- The lack of pepsin activity – protein is only partly degraded in accordance to substrate specificity of pepsin
- The lack of trypsin activity (and other of pancreatic origin) – there are no aminoacids for intestine absorption
- the lack of exogenous aminoacids for protein synthesis (alterations in hair, fur, muscles), biologically active substances

Hormones:

Hormones taking part in protein digestion

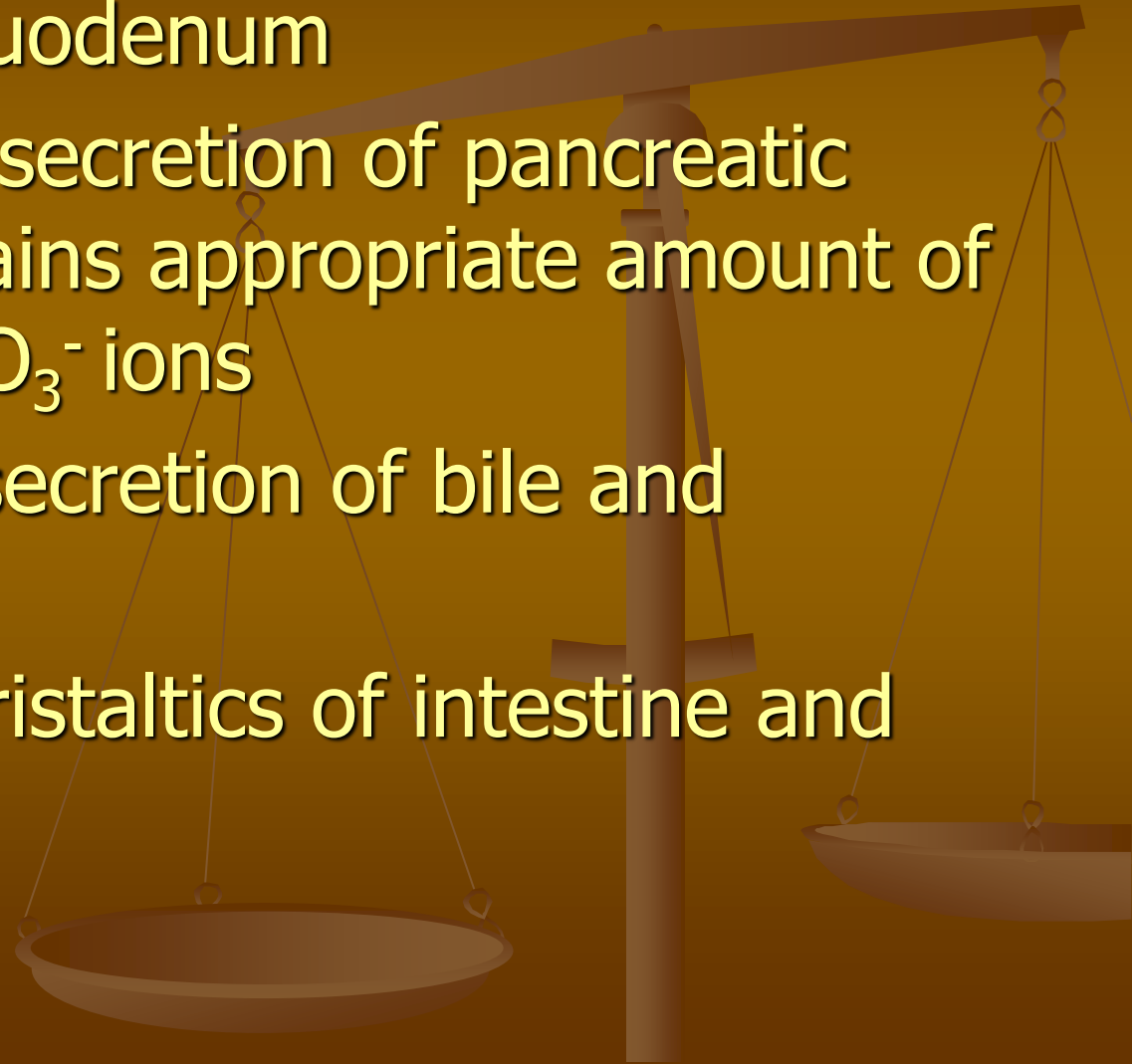
Gastrin

- ✓ Stimulates the secretion of HCl
- ✓ Stimulates the secretion of stomach juice
- ✓ Increases the peristaltics of digestive tract
- ✓ Has a trophic effect on the mucous membrane of stomach
- ✓ Increases blood flow in intestines
- ✓ Secretion is inhibited by:
 - The decrease of pH below 2,5
 - Secretin
 - Somatostatin

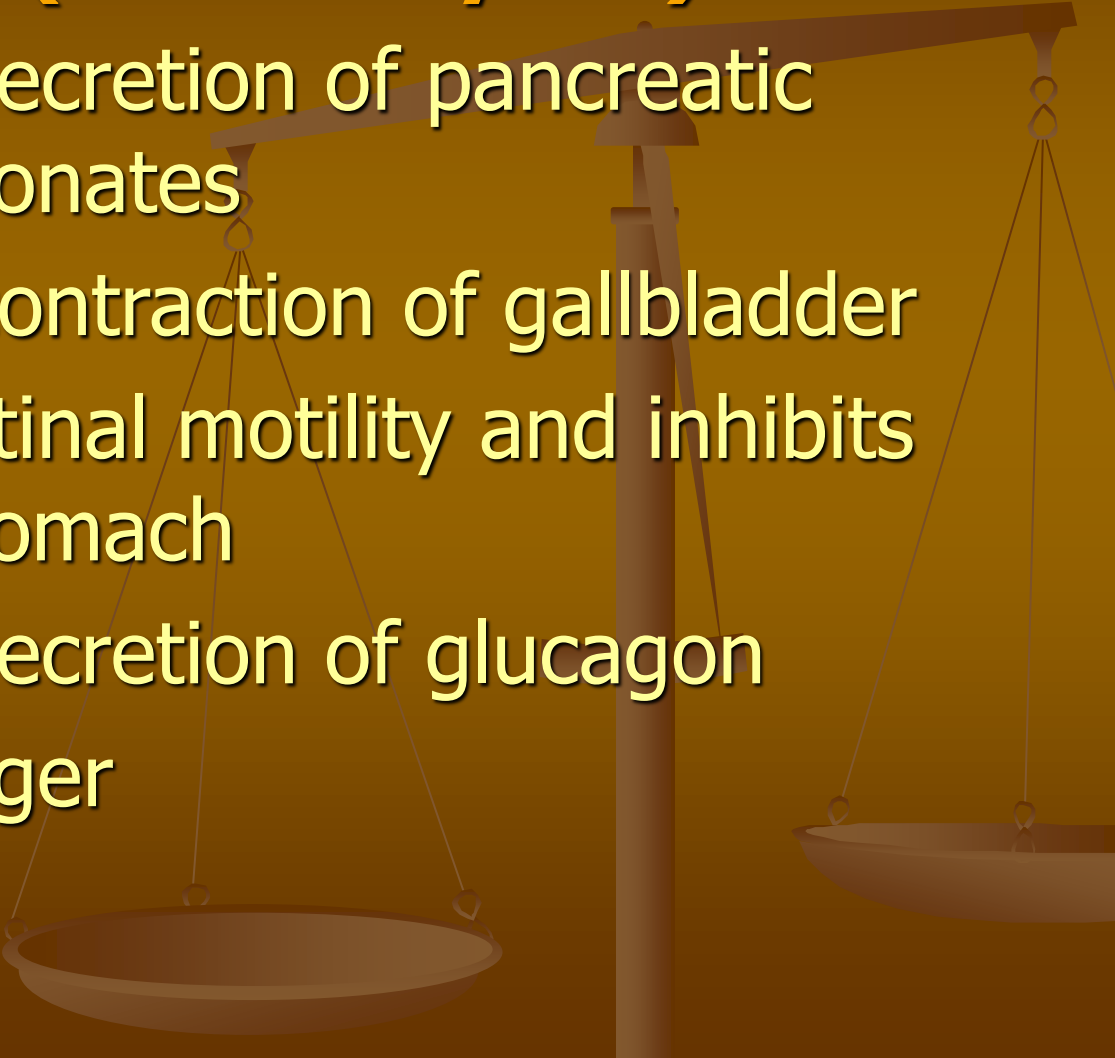


Secretin

- ✓ Secreted under the influence of acidic pH coming from duodenum
- ✓ Stimulates the secretion of pancreatic juice that contains appropriate amount of water and HCO_3^- ions
- ✓ Increases the secretion of bile and intestine juice
- ✓ Inhibits the peristaltics of intestine and stomach

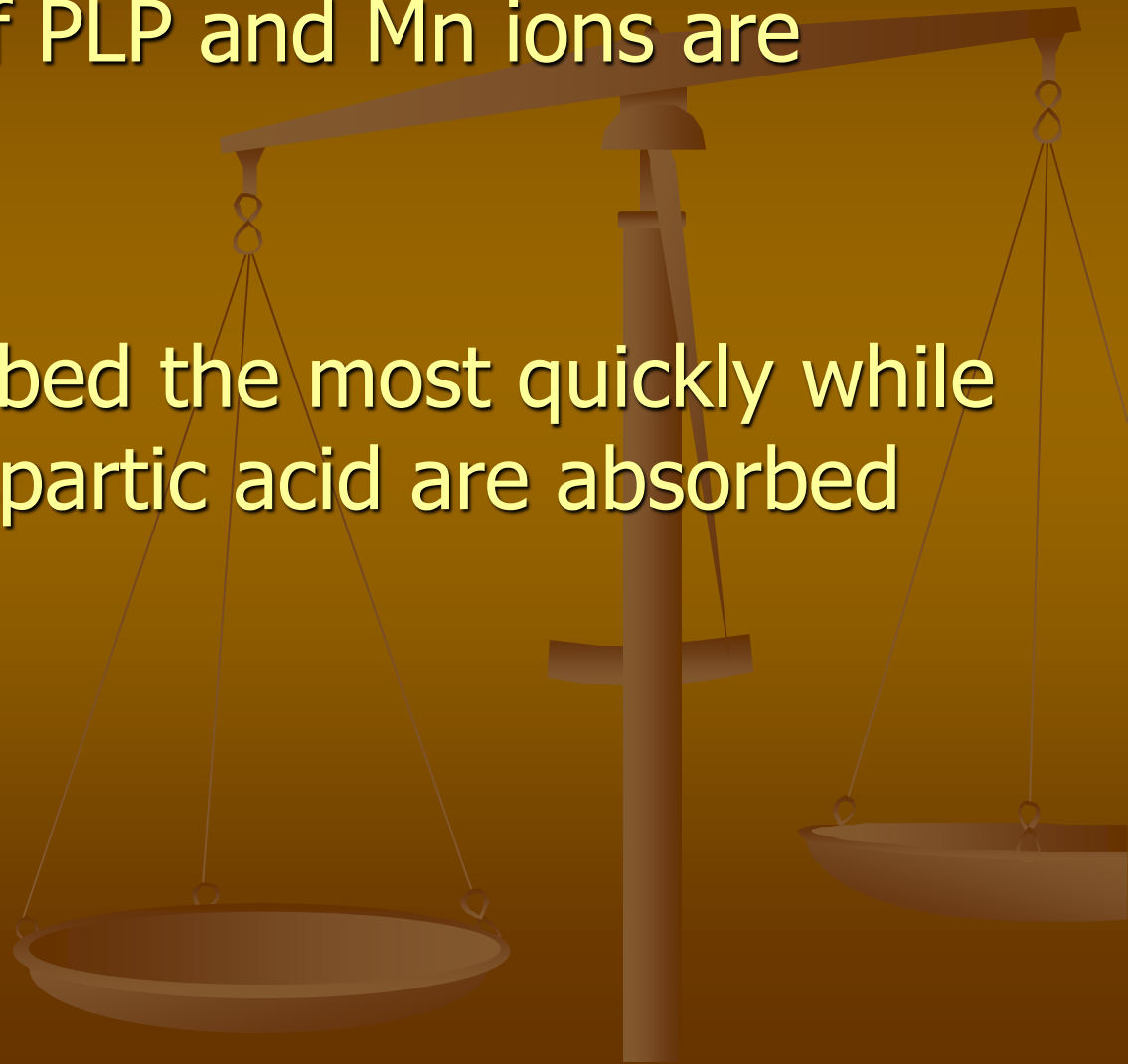


Cholecystokinin (Pancreosymin)

- ✓ Stimulates the secretion of pancreatic juice and bicarbonates
 - ✓ Stimulates the contraction of gallbladder
 - ✓ Stimulates intestinal motility and inhibits peristaltics of stomach
 - ✓ Stimulates the secretion of glucagon
 - ✓ Inhibits the hunger
- 

Absorption of aminoacids:

- The presence of PLP and Mn ions are indispensable
- Cystein is absorbed the most quickly while glutamic and aspartic acid are absorbed the most slowly



Experiment 1

The examination of pepsin activity in different conditions of pH

Protocol: pipete 2 cm³ of pepsin to tubes marked 1, 2, 3, 4.
Tube no 4 heat over the burner (inactivation of pepsin).

Add the following solutions to appropriate tubes:

- 1 - 2 cm³ 0,2 mol/dm³ HCl
- 2 - 2 cm³ 1 mol/dm³ CH₃ COOH
- 3 - 2 cm³ buffer pH 7,2

Add small amount of protein to all 4 tubes. Incubate tubes in water bath for 1 hour at 37°C. Observe the tubes after incubation and give the number of tube where protein was digested. Explain obtained results.

Experiment 2

The determination of trypsin activity

Protocol: Add 1 cm³ of 30% TCA to three centrifuge tubes and mark them 1, 2, and 3. In glass tube marked „A” prepare incubation mixture containing 4 cm³ of 1% casein in 0,1 mol/dm³ NaHCO₃ and 1 cm³ of trypsin solution. Mix the content carefully and immediately take 1 cm³ of this mixture to centrifuge tube marked 1.

The remaining mixture of tube „A” incubate during 10 min. in temp. 37°C. After 10 min. take 1 cm³ of this mixture and add to centrifuge tubes 2 and 3.

Leave tubes 1, 2, 3 in room temperature for 10 min. and centrifuge them for 15 min. at 2000xg. Remove the supernatant and add 1 cm³ of 1 mol/dm³ NaOH to each tube to resolve the precipitate. After resolving the precipitate add 4 cm³ of cooper reagent to each tube. Mix the solution and leave it in room temperature for 20 min.

In the meantime prepare blank sample by mixing 4 cm³ of cooper reagent and 1 cm³ of distilled water. Read the absorbance of samples 1, 2, 3 at wavelength 540 nm against blank sample. The content of protein in examined solution can be estimated based on standard curve.