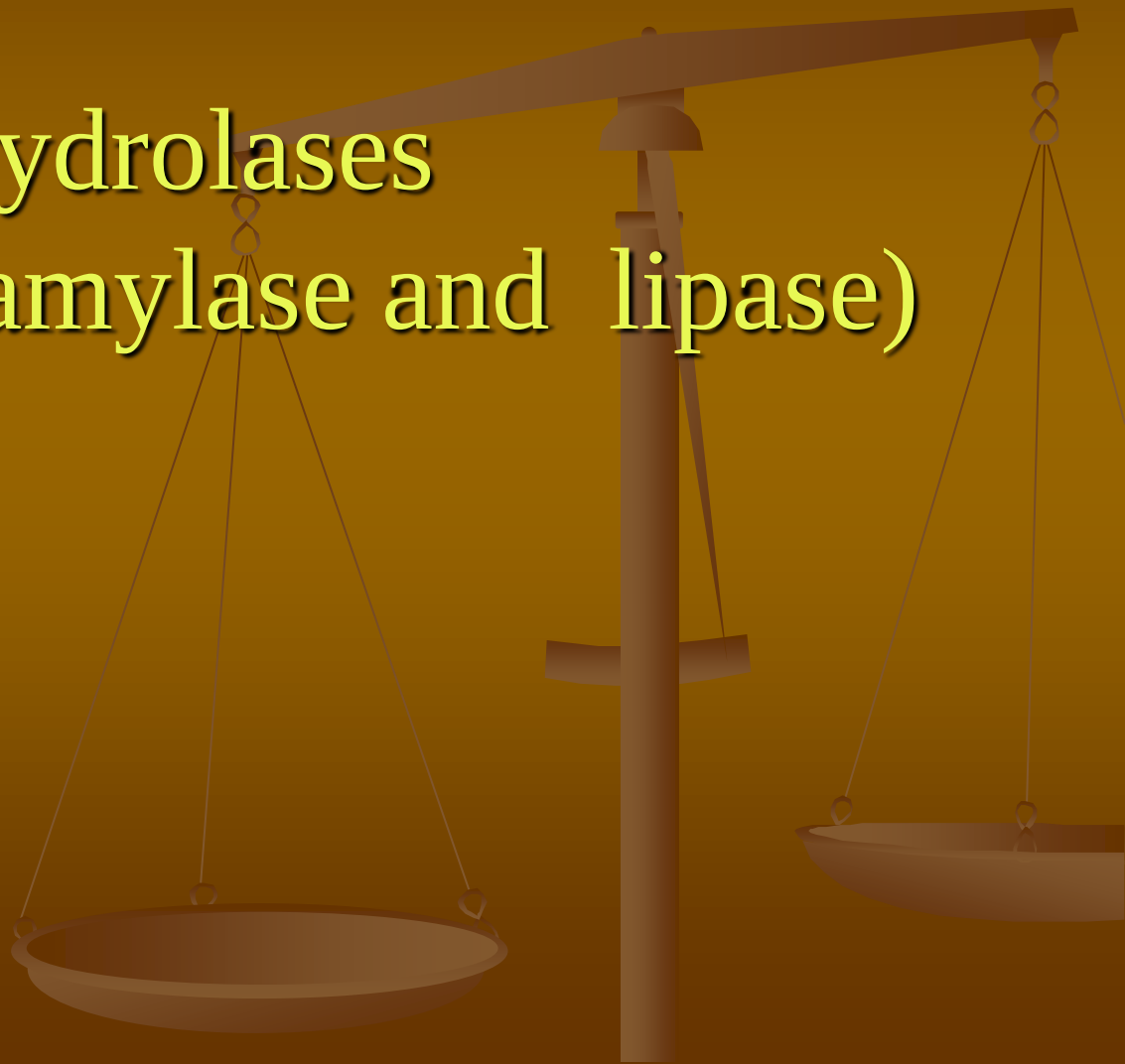
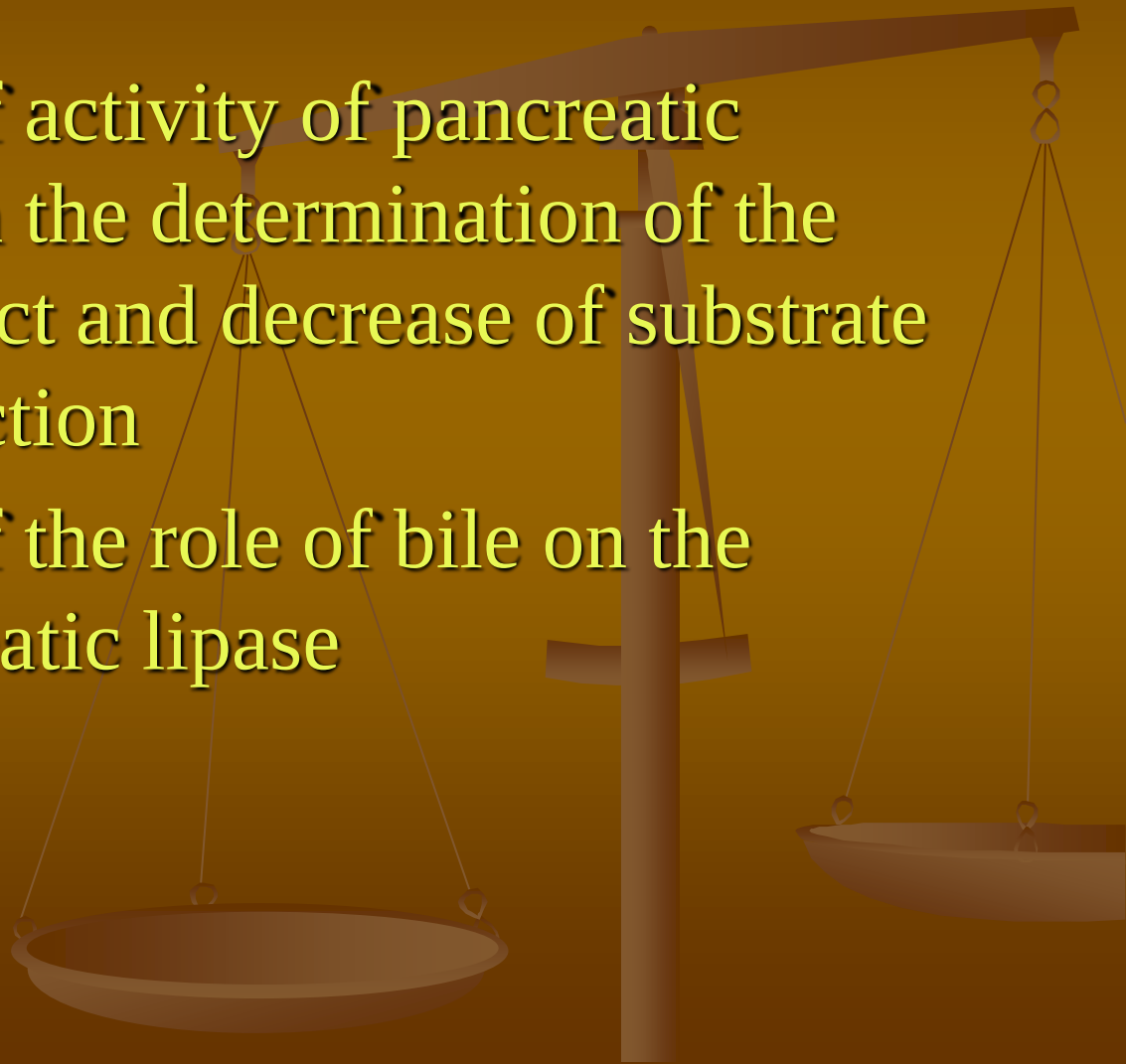
Hydrolases
(pancreatic amylase and lipase)



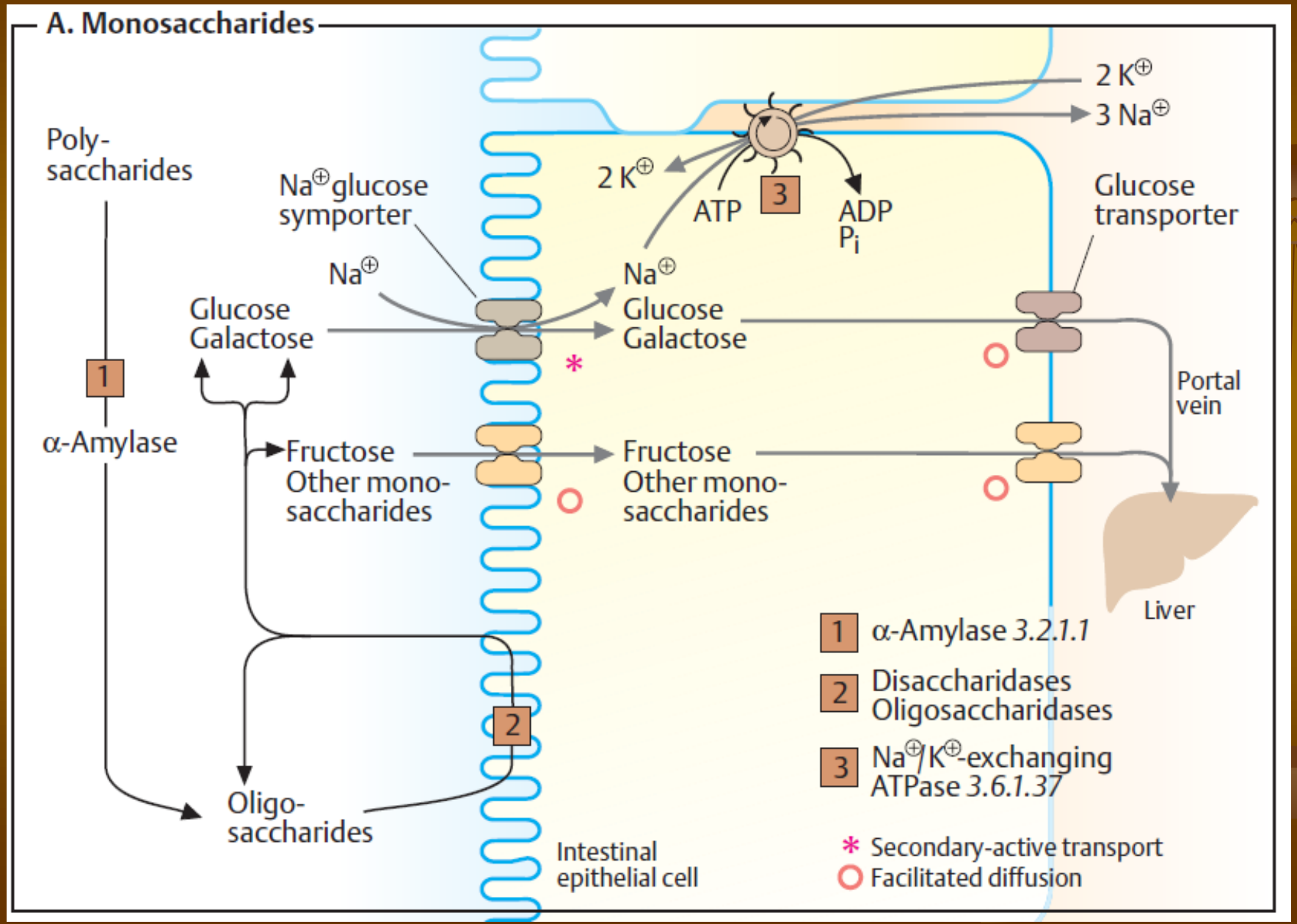
E.C.3.2.1.1.
pancreatic Alfa-amylase

The aim:

- The estimation of activity of pancreatic amylase based on the determination of the increase of product and decrease of substrate of enzymatic reaction
- The estimation of the role of bile on the activity of pancreatic lipase



Amylolytic enzyme of pancreatic juice



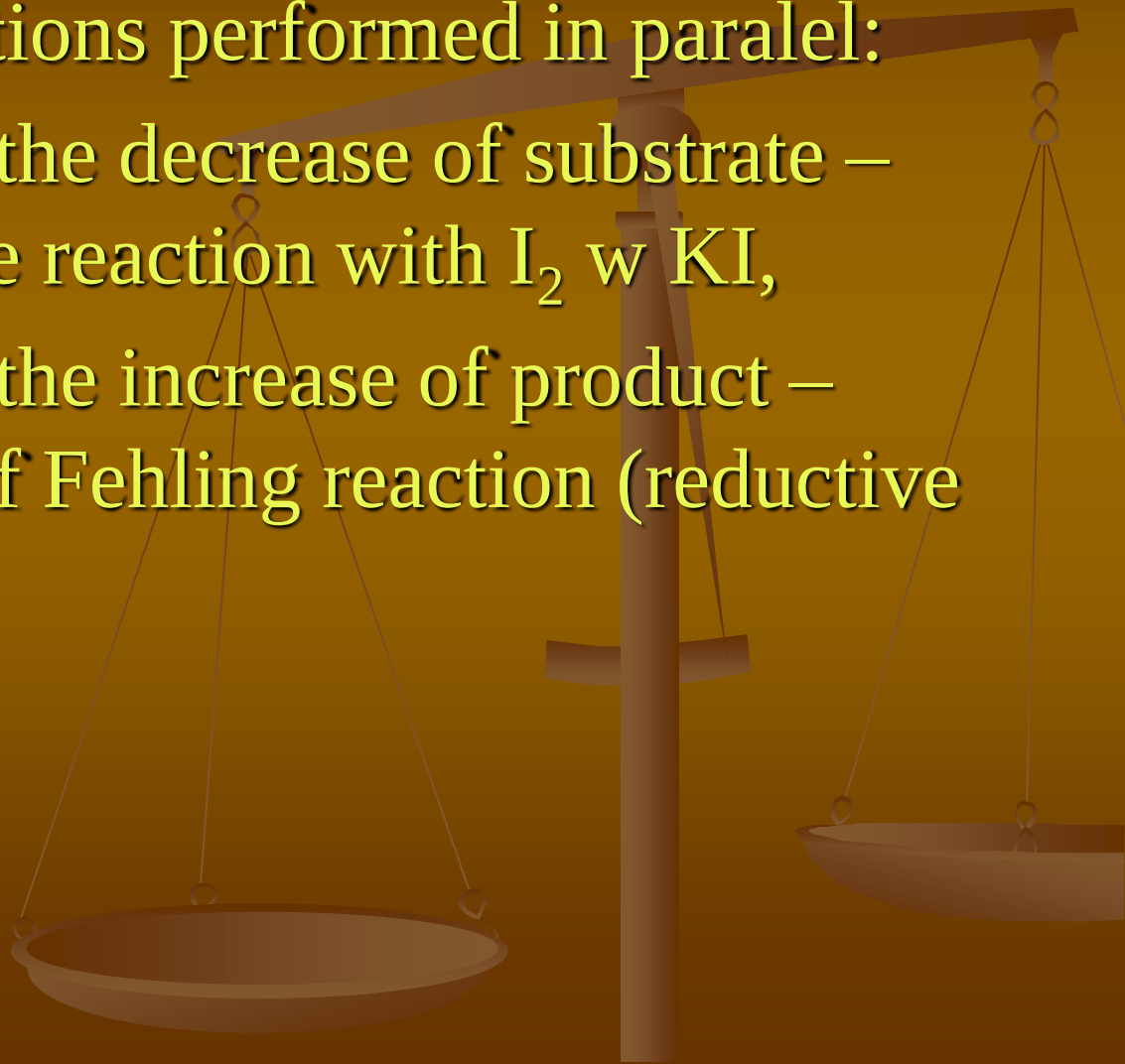
Mechanism of action:

- Optimum pH 6,7-7,2
- Cl ions act as activators
- Enzyme hydrolyses alfa1,4-glycosidic bonds in starch
- Enzyme is not active on alfa1,6-glycosidic bonds
- The products of reaction are maltose and isomaltose

The determination of activity:

Is based on two reactions performed in paralel:

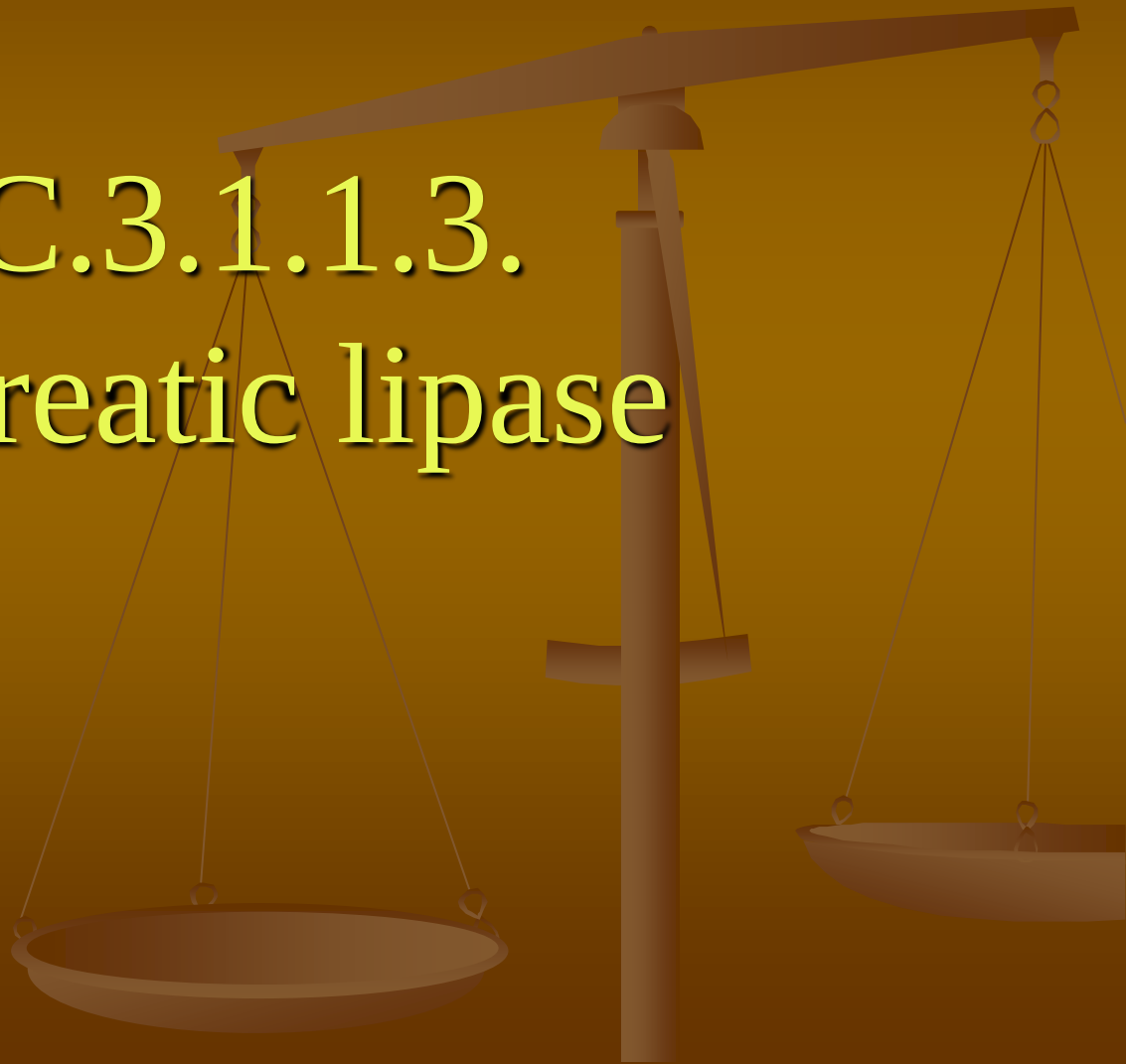
1. The estimation of the decrease of substrate – starch by use of the reaction with I_2 w KI,
2. The estimation of the increase of product – maltose – by use of Fehling reaction (reductive reaction)



Reaction with iodine

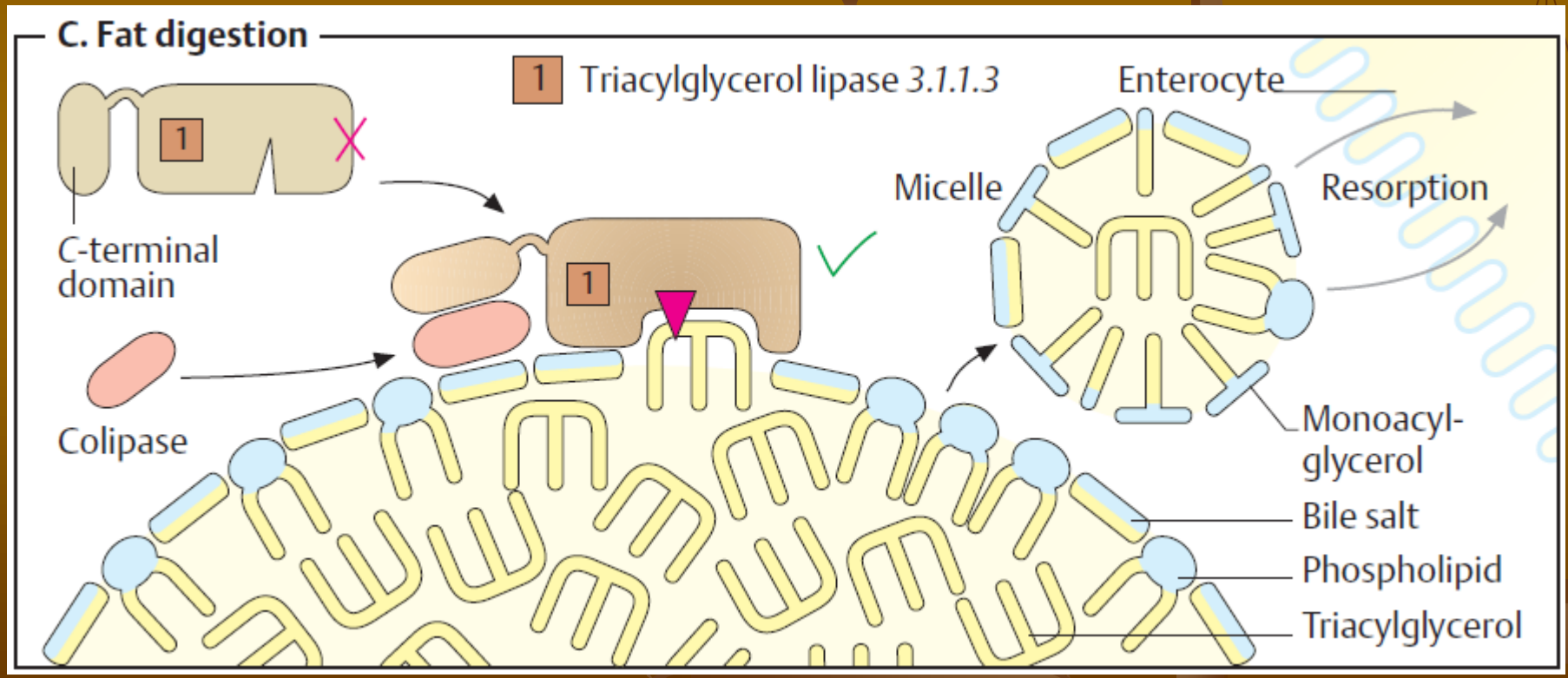
- The staining of some polysaccharides under the influence of iodine is based on the adsorption of iodine molecules on molecules of polysaccharide. This adsorption is of channel character – molecules of iodine are incorporated into channels created by spirally turned chains of sugars. Molecules of iodine within the helix of sugar create straight chain of iodine along which electrons can be transferred. It causes the absorption of light via whole complexes. Amylose gives blue colour, amylopectin and amylodextrins – violet while erythrodextrins and glycogen red colour.

E.C.3.1.1.3. Pancreatic lipase

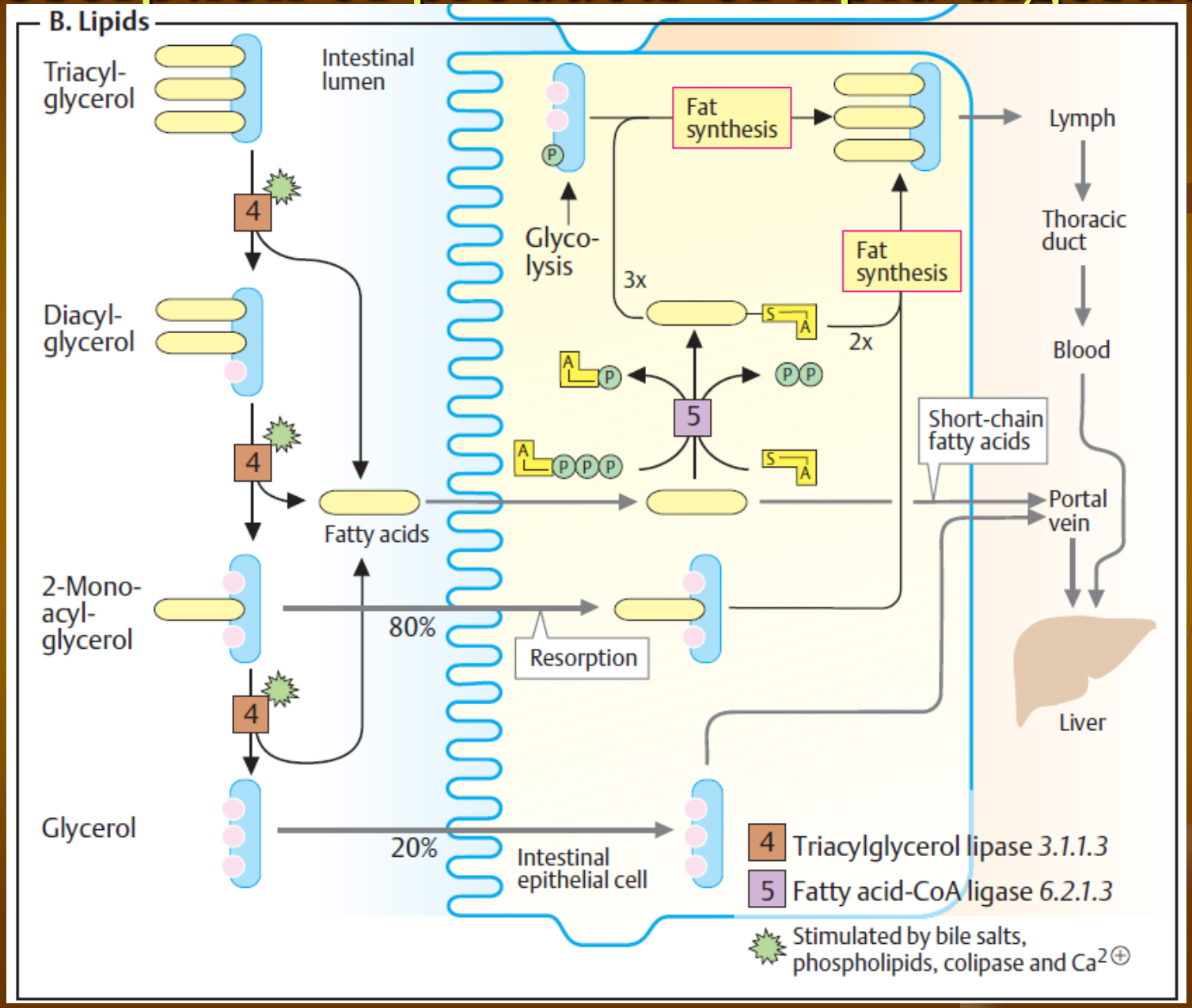


Lipolytic enzyme of pancreatic juice.

Is synthesised as inactive proenzyme.



Absorption of products of lipid digestion

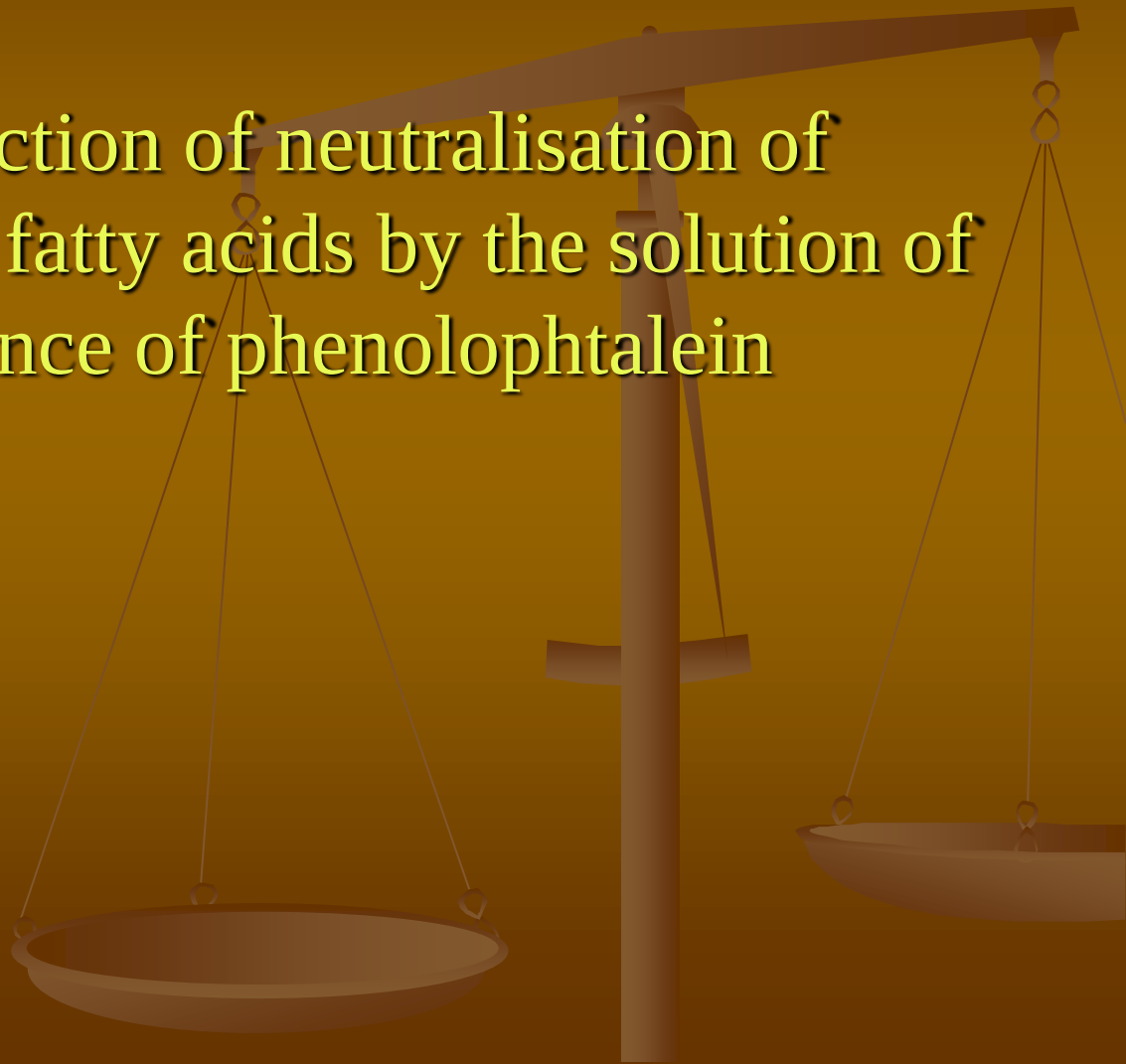


Mechanism of action:

- Optimum pH 7-8,5
- The presence of bile is indispensable (bile acids cause the emulgation via the decrease in surface pressue)
- Cofactors of enzyme are calcium ions and colipase (protein)
- It catalyses hydrolytic breakdown of ester bonds of lipid into glycerol and fatty acids
- The process is performed step by step – due to specific preferences in selection of ester bonds - alfa, alfa’ and beta

The determination of activity:

Is based on the reaction of neutralisation of liberated by lipase fatty acids by the solution of NaOH in the presence of phenolophtalein



Experiment 1

The aim of the task is to determine α-amylase activity in the pancreatic extract.

Protocol

Add 5 cm^3 of 0.5% starch solution and 1 cm^3 of pancreatic extract to the test tube using a pipette (to obtain a working solution). Put the probe in water bath at 37°C.

After the time periods specified in the table follow analysis and note results:

a. Fehling's test for reducing sugars. For this purpose mix 0.5 cm^3 Fehling I and Fehling II solution and 0.5 cm^3 of working solution. Carefully heat working solution over the burner for a few minutes.

b. 0.001 mol/dm^3 J_2 in potassium iodide(KJ)to detect the presence of a starch. For this purpose pour 10 drops of J_2 in KJ into the 0.5 cm^3 of working solution.

Number of probe	Incubation period [min]	Test for reducing sugars	Iodine test
1	0		
2	5		
3	10		
4	20		
5	30		

Experiment 2

The aim of the task is to determine lipase activity in the pancreatic extract.

Protocol

Add 1 cm^3 of 0.1 mol/dm^3 Na_2CO_3 to the 15 cm^3 of oil and shake it vigorously to obtain oil-in-water emulsion. Place the appropriate amounts of pancreatic extract, water, oil-in-water extract and bile to the 3 test tubes, according to the table.

Number of probe	Pancreatic extract [cm^3]	Water [cm^3]	Oil-in water emulsion [cm^3]	Bile [cm^3]	0.05 mol/dm^3 NaOH [cm^3]
1	2	1	3	0	
2	2	0.75	3	0.25	
3	-	2.75	3	0.25	

Probe 1 – probe „0", probe 3 – control probe relative to probe 2.

Incubate all test tubes for 60 min (37°C). During incubation shake the tubes every 5 minutes. After finishing the incubation pour solutions from the tubes into the small Erlenmeyer flask. Add 3 drops of phenolphthalein to each flask and titrate with 0,05 mol/dm^3 NaOH until the permanent pink color (fuchsia).

Enter the results in the table. Explain.