SL Dr. Corina Samoilă corisamoila@umft.ro
As. Dr. Anda Alexa alexa.anda@umft.ro
All questions will be addressed to the corresponding laboratory teaching assistant and guidance will be provided.

L6. Lipid metabolism. Determination of triglycerides. Identification of ketone bodies in urine

Various types of lipids occur in the human body:

- Triacylglycerol
- Cholesterol
- Polar lipids (phospholipids, glycolipids and sphingolipids).

In contrast to polar lipids and cholesterol, which are found in the membranes of every cell, triacylglycerol is concentrated mostly in adipose (fat) tissue; minor amounts of triacylglycerol occur in other cell types, such as liver epithelia and skeletal muscle fibers. Triacylglycerol is the most abundant lipid species, and the only one with an important role in energy metabolism. Triacylglycerol occurs in human metabolism in two roles: as a foodstuff, which accounts for a significant fraction of our caloric intake, and as astore of metabolic energy.

Digestion

Dietary triacylglycerol undergoes hydrolysis in the digestive tract. The main products of hydrolysis are monoacylglycerol and free fatty acids. The fatty acids found in natural fats vary both in chain length and in the number of double bonds.

While most of the dietary triacylglycerol is hydrolyzed by pancreatic triacylglycerol lipase in the small intestine, fat digestion is already initiated by *gastric* lipase, which is released by the mucous membrane of the stomach.

After solubilization and lipase digestion, monoacylglycerol and free fatty acids are taken up by epithelial cells in the mucous membrane of the small intestine. What happens to them once inside is somewhat surprising: they are immediately converted back to triacylglycerol. This involves the transient activation of fatty acids to acyl-CoA at the expense of ATP. The newly formed fat is then combined with protein molecules called *apolipoproteins* into lipoprotein particles, such that the proteins form a hydrophilic shell around the lipid core. Some phospholipids are included as well and complete the hydrophilic shell.

Lipoproteins occur in various subtypes. The specific type formed at this stage, the *chylomicrons*, are the largest of all lipoproteins. They transport dietary lipids from the intestines to other locations in the body. Chylomicrons are one of the five major groups of lipoproteins: chylomicrons, very low-density lipoprotein, intermediate-density lipoprotein, low-density lipoprotein, high-density lipoprotein, that enable fats and cholesterol to move within the water-based solution of the bloodstream.

DETERMINATION OF SERUM TRIACYLGLYCEROLS

Introduction

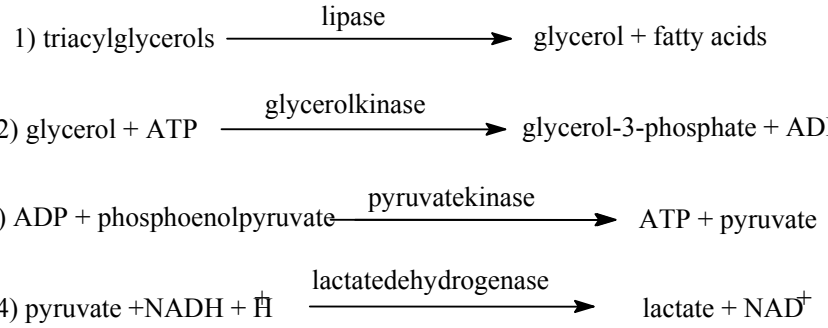
Triacylglycerols, also called neutral fats, do not give a specific recognition method. That is why their determination can be done using an indirect method, by calculation, or a direct method in which just one part of their molecule is determined, calculating finally their total concentration.

The determination of serum or plasma triacylglycerol concentration is used to evaluate and differentially diagnose the primary or secondary hyperlipidemias, and for the evaluation of the risk factors of acute pancreatitis.

The methods used nowadays determine the glycerol from the triacylglycerol molecule, determination that can be done using chemical or enzymatic methods.

All the enzymatic methods have, at base, a sequence of enzymatic reactions, each one using a specific enzyme, characteristic for that method.

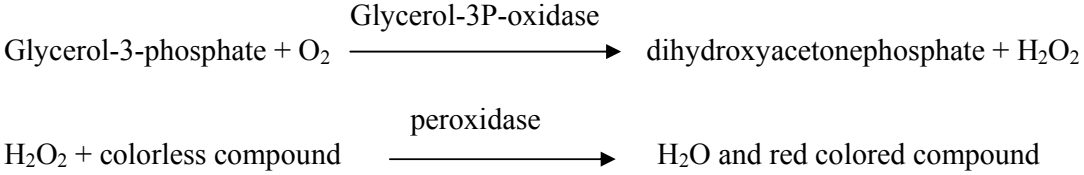
The technique with pyruvate kinase, having a final measurement in ultraviolet, has the following sequence of reactions:



The decrease of absorbency at 340 nm is directly proportional to the level of serum triacylglycerols.

The techniques that use glycerolphosphate oxidase have the following sequence of reactions:

The first two reactions are identical with the method presented above, then follows:



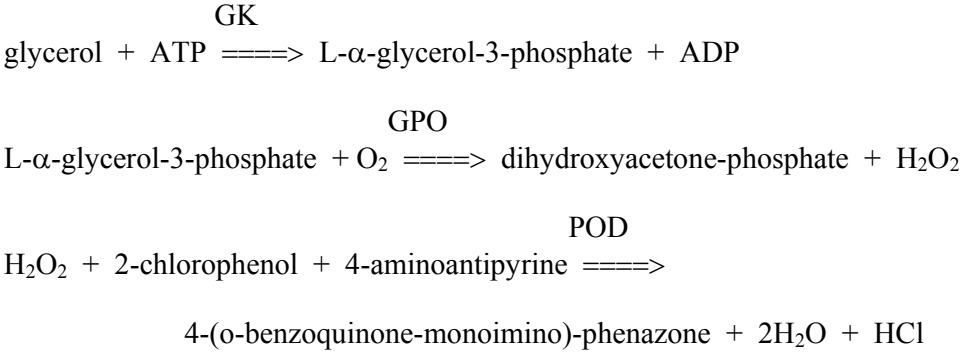
The colour intensity is directly proportional to the triacylglycerols level.

Experimental part

Serum triacylglycerol determination with the enzymatic method

Principle

Triglycerides are enzymatically hydrolyzed to glycerol and free fatty acids in the presence of specific lipases. Glycerol reacts further according to the following reaction scheme:



where:
GK - glycerokinase,
GPO - glycerol phosphate oxidase,
POD - peroxidase

Procedure

Reagents (µl)	Sample	Standard	Blank
Serum	100	-	-
Standard (200 mg/dl)	-	100	
Distilled water	-	-	100
Reactive mixture	1000	1000	1000

Mix and incubate 10 min. at room temperature. Read the sample and standard absorbance at 546 nm zeroing the spectrophotometer against the blank.

Calculation

To calculate the serum triglyceride concentration use the formula:

$$\textbf{mg\% triacylglycerol} = (E_S/E_{St}) \times 200$$

Normal values

Women: 40-140 mg%
Men: 60-165 mg%

Pathological values

Increased values are found in: primary hyperlipoproteinemias (exception type IIa), myocardial infarct, diabetes mellitus, obesity, hypothyroidism, hepatic diseases, obstructive jaundice, nephritic syndrome, pregnancy, cortisol and estrogens treatment.

Decreased values are found in: severe anemia, consumptive diseases, starvation.

KETOGENESIS

Ketogenesis is a pathway of acetyl-CoA utilization, when it is in excess, to form ketone bodies:

- acetoacetate
- β-hydroxybutirate
- acetone

The concentration of total ketone bodies in the blood of well-fed mammals does not normally exceed 0.2 mmol/liter (1 mg%) Loss via the urine is usually less than 1 mg/24 h in humans.

In vivo, the liver appears to be the only organ in non-ruminants to add significant quantities of ketone bodies in the blood. Extra-hepatic tissues utilize them as respiratory substrates (for energy production).

The net flow of ketone bodies from the liver to the extra-hepatic tissues results from an active enzymatic mechanism of the liver (into the mitochondria) for the production of ketone bodies coupled with very low activity of enzymes responsible for their utilization. The reverse situation occurs in extra-hepatic tissues.

Higher than normal quantities of ketone bodies present in the blood (up to 60-70 mg%) or in urine constitutes ketonemia (hyperketonemia) or ketonuria, respectively. The overall condition is called ketosis. Acetoacetic and β-hydroxybutiric acids are both moderately strong acids and are buffered when present in blood or other tissues. However, their continual excretion in quantity entails some loss of buffer cations (in spite of ammonia production by the kidney) that progressively depletes the alkali reserve, causing ketoacidosis. This may be fatal in uncontrolled diabetes mellitus.

The simplest form of ketosis occurs in starvation, and involves depletion of available carbohydrate coupled with mobilization of free fatty acids.

Quantitatively it may be exaggerated to produce the pathologic states found in diabetes mellitus, pregnancy toxemia in sheep, and ketosis in lactating cattle.

Non pathologic forms of ketosis are found under conditions of high fat feeding and after severe exercise in the post absorptive state. Hepatic tissue, in the presence of fatty acids in excess, produces large quantities of ketone bodies.

Experimental part

Ketone bodies identification in urine using the Legal-Imbert method

Principle

Legal-Imbert method is used to feature acetoacetic acid and acetone. Ketone bodies from urine form, with nitropruside and ammonia, a violet colored ring.

Procedure

Reagents	**Pathologic**
Urine	2.0 ml
Legal-Imbert reagent, drops	5-6 drops
Ammonia 25%	7-8 drops

Do not mix. Two liquid layers must appear. The formation of a violet ring at the contact between the two layers indicates the presence of the ketone bodies.

Normal urine will not form the violet ring.