4. LIPIDS

Biological lipids are a chemically diverse group of compounds, the common and defining feature of which is their insolubility in water, but soluble in an organic solvent (e.g., ether, benzene, acetone, chloroform).

The biological functions of the lipids:

(1) Fats and oils are the principal stored forms of energy in many organisms.

(2) Phospholipids and sterols are major structural elements of biological membranes.

(3) Maintenance of temperature: Layers of subcutaneous fat under the skin also help in insulation and protection from cold.

(4) Mechanical protection

(5) Electrical insulation of nerves

(6) Wax coating that protects plants

(7) Other lipids, although present in relatively small quantities, play crucial roles as;

- enzyme cofactors,
- electron carriers,
- light absorbing pigments,
- hydrophobic anchors for proteins, "chaperones" to help membrane proteins fold,
- emulsifying agents in the digestive tract,
- hormones, and
- intracellular messengers

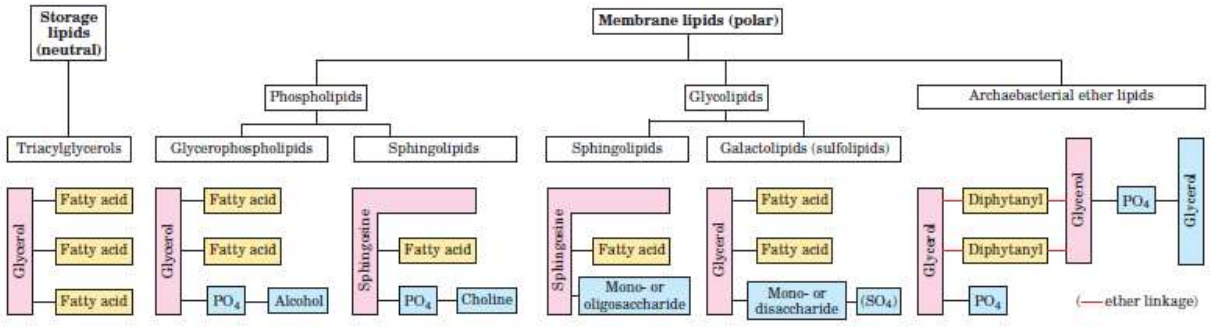

Figure 4.1 Some common types of storage and membrane lipids.

4.1 Triacylglycerols (TAG)

A **triacylglycerol** (or **triglyceride TG**, **triacylglyceride**) is an ester derived from glycerol and three fatty acids (from *tri-* and *glyceride*). The glycerol molecule has three hydroxyl (HO–) groups. Each fatty acid has a carboxyl group (–COOH). In triglycerides, the hydroxyl groups of the glycerol join the carboxyl groups of the fatty acid to form ester bonds. The simplest triglycerides are those where the three fatty acids are identical. They rarely occur in nature. Instead, a typical triglyceride obtained from naturally occurring fats and oils contains two or three different fatty acid components and is thus termed a mixed triglyceride. Triacylglycerols are the form in which fat energy is stored in adipose tissue. The various dietary plant oils, such as olive oil and corn oil, are also triacylglycerols. The presence of unsaturated fatty acids in a triacylglycerol molecule makes it more fluid. This is due to the presence of the kinks in unsaturated fatty acids, which keeps the fatty acid chains from aligning uniformly. This is why plant oils, which contain primarily unsaturated fatty acids, are more liquid than animal fats.

Triglycerides can be hydrolyzed in the presence of an acid, a base, or specific enzymes known as lipases. The hydrolysis of fats and oils in the presence of a base is used to make soap and is called saponification.

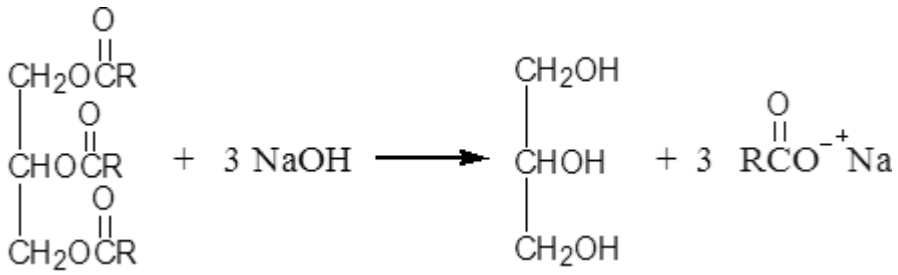

Figure 4.2 The hydrolysis of fats

4.2 Cholesterol

Cholesterol, the major sterol in animal tissues, is amphipathic, with a polar head group (the hydroxyl group at C-3) and a nonpolar hydrocarbon body (the steroid nucleus and the hydrocarbon side chain at C-17), about as long as a 16- carbon fatty acid in its extended form.
Cholesterol, given that it composes about 30% of all animal cell membranes, is required to build and maintain membranes and modulates membrane fluidity over the range of physiological temperatures. In addition to its importance for animal cell structure, cholesterol also serves as a precursor for the biosynthesis of steroid hormones, bile acid and vitamin D. Since all animal cells

manufacture cholesterol, all animal-based foods contain cholesterol in varying amounts. Major dietary sources of cholesterol include cheese, beef, fish, poultry and shrimp. Human breast milk also contains significant quantities of cholesterol. Body makes all the cholesterol it needs. Atherosclerosis is a vascular disease. Atherosclerosis is fairly common, especially as we age. It happens when fat and cholesterol in the blood form plaque which can build up inside the artery walls. If this plaque begins to block the arteries, it can slow and limit the flow of blood to your organs. When this happens it can cause serious problems in blood vessels throughout the body, including heart attack, stroke, peripheral arterial disease and kidney disease.

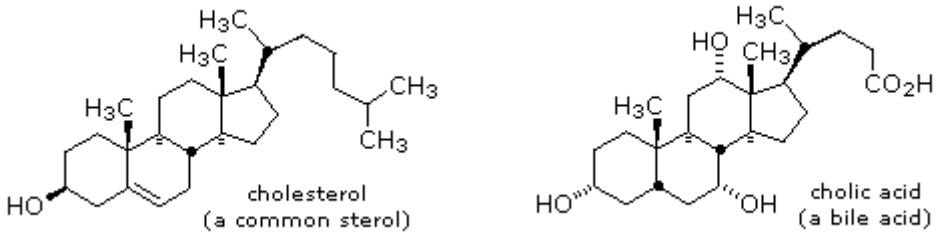

Figure 4.3 Formulas of cholesterol and bile acid

4.3 Ketone Bodies

Ketone bodies are produced from acetyl-CoA, mainly in the mitochondrial matrix of liver cells when carbohydrates are so scarce that energy must be obtained from breaking down of fatty acids. The three ketone bodies are acetoacetate, β-hydroxy butyrate and acetone. when excess ketone bodies accumulate, this abnormal (but not necessarily harmful) state is called ketosis. Ketone body synthesis occurs normally under all conditions. However, the formation of ketone bodies increases dramatically during starvation and in diabetes (is a disorder where the body does not produce insulin or does not use it efficiently). Prolonged low levels of insulin result in both increased fatty acid release from adipose tissue, and increased amounts of the enzymes required to synthesize and utilize ketone bodies. In addition, in the liver, increased demand for gluconeogenesis results in depletion of oxaloacetate, and therefore in decreased capacity for the TCA cycle. This causes a rise in the levels of acetyl-CoA, the substrate for ketone body production. When even larger amounts of ketone bodies accumulate such that the body's pH is lowered to dangerously acidic levels, this state is called ketoacidosis.

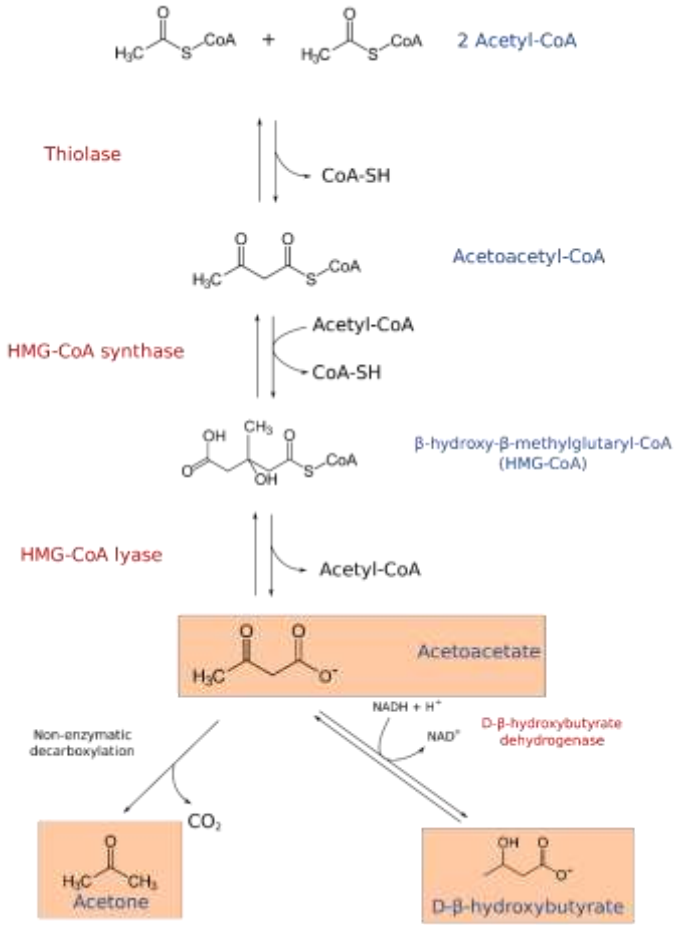

Figure 4.4 The synthesis of ketone bodies

4.4 Experiments

Saponification is a process that involves conversion of fat or oil into soap and alcohol by the action of heat in the presence of aqueous alkali (e.g. NaOH). The triglyceride is treated with a strong base, which cleaves to the ester bond, releasing fatty acid salts (soaps) and glycerol.

4.4.1 Hydrolysis of Corn Oil

Corn oil consists of a 15% saturated fatty acid and 85% unsaturated fatty acid. Even though it contains high levels of unsaturated fatty acids, it only contains 0.1% tocopherol, so it is resistant to autooxidation. The fatty acids of corn oil can be hydrolyzed to get an average molar mass. Fatty acids are separated from glycerol and salts after base hydrolysis in the presence of alcohol as a result of the following reactions. The fatty acid in the sample is based on the calculation of the molar mass by titration with NaOH.

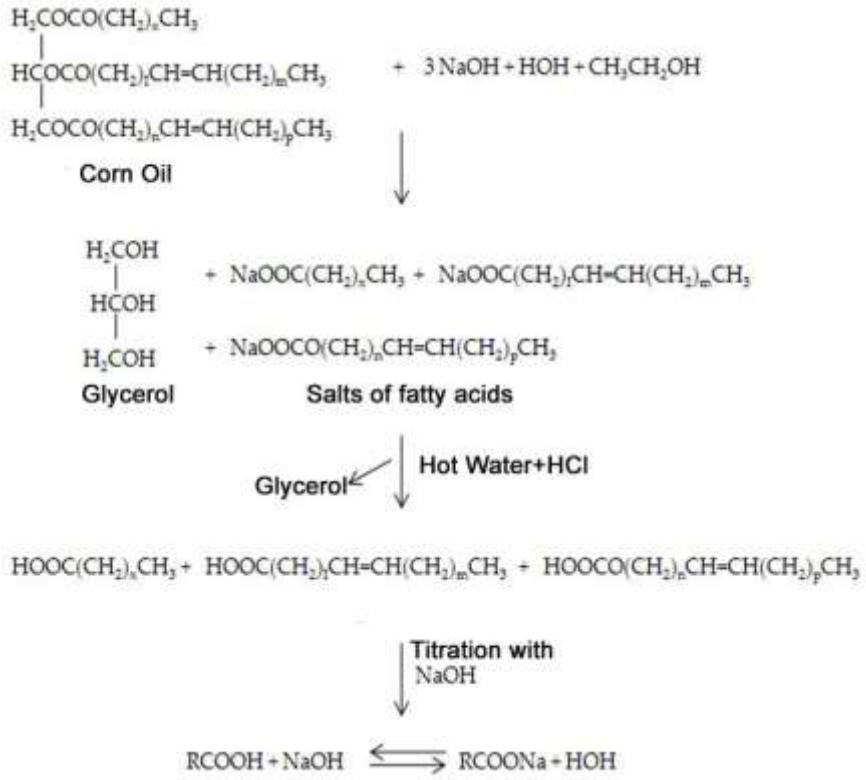

Figure 4.3. Hydrolysis of corn oil

Principle of the experiment: The free fatty acids in corn oil are measured by extracting the sample with hot ethyl alcohol and titrating the supernatant alcohol layer with standard alkali.

Experimental procedure

(1) Weigh about 0.25 g of corn oil sample into a 50 mL Erlenmeyer flask. Add 25 mL of hot, neutralized ethyl alcohol (with Phenolphthalein indicator, 1%). Heat the mixture to near boiling and titrate while hot with 0.1 N sodium hydroxide solution to the first permanent pink color. The pink color should be observed in the alcohol layer above the sample for a few seconds.

(2) The molar mass of the oil is calculated by determining the volume of NaOH spent in titration.

The average molar mass is calculated from the formula:

$$M_{avg} = \frac{Weight\ of\ sample\ (g)}{V_{base} \times N_{base}}$$

4.2.2 Determination of Acid Value (Acid Number) in Lipids

The acid value is often a good measure of the break down of the triacylglycrols into free fatty acids, which has an adverse effect on the quality of many lipids. The acid value (*AV) is* the number that expresses, in milligrams the quantity of potassium hydroxide required to neutralize the free acids present in 1 g of the substance. Acceptable levels for all oil samples should be below 0.6 mg KOH/g.

Principle of the experiment: The free fatty acid in oil is estimated by titrating it against KOH in the presence of phenolphthalein indicator. The acid number is defined as the mg KOH required to neutralize the free fatty acids present in 1g of sample.

Experimental Procedure

(1) Take 0.5 mL of oil in a erlenmeyer flask and dissolve in 15 mL of a mixture of equal volumes of alcohol and ether 1:1 (v/v).

(2) After dissolving procedure, a spatula-tip phenolphthalein is added the above solution. Titrate the solution against 0.1 N potassium hydroxide or 0.1 N sodium hydroxide Shake constantly until a pink color which persists for 5 seconds is obtained. The volumes of the titrant consumed are noted (V1).

(3) Blank Solution: Take 15 mL of a mixture of equal volumes of alcohol and ether 1:1 (v/v) in a erlenmeyer flask. Put a small amount (spatula-tip) of substance phenolphthalein in the solution. The solution titrate with 0.1 N potassium hydroxide or 0.1 N sodium hydroxide (without phenolphthalein). The volumes of the titrant consumed are noted (V2).

$$number\ of\ acids\ (Ax) = \frac{V_1 - V_2}{\mathrm{W}} \times 56.11 \times N$$

4.4.3. Test for free fatty acids

Principle of the experiment: When the phenolphthalein is placed in a basic solution such as 0.1 N sodium hydroxide it appears pink in colour. However, free fatty acids in oils as a hydrogen ion content of the oil solution increases, the phenolphthalein fades to colourless.

Experimental Procedure

(1) Dilute NaOH (0.1 M) solution and phenolphthalein solution are carefully mixed until a permanent pink color is obtained in a tube.

(2) Butter (a spatula-tip), olive oil (2 mL) and oleic acid (2 mL) are dissolved in three separate tubes by adding 2 mL ether.

(3) An alkaline phenolphthalein solution prepared is added drop by drop to these tubes and colour changes are observed.

Qualitative and Quantitative Determination of Cholesterol

4.4.4. Determination of Cholesterol by Salkowski Method

Principle of the experiment: Sterols are alcohols, the Salkowski test involves treating sterols like cholesterol with a strong acid which leads to the protonation of the hydroxyl groups of the alcohol. Colour change occurs due to the formation of charge transfer complexes in the resulting conjugated dienes.

Experimental Procedure

Put 2 mL of chloroform into a test tube, a spatula tip cholesterol is added to the tube. Add carefully 2 mL of concentrated H_2SO_4 onto the solution. Red ring and light green colour are observed at the contact point of the formed layers.

4.4.5 Quantitative Determination of Cholesterol by Liebermann - Burchard Test

Principle of the experiment: It is based on the fact that the cholesterol released by precipitation of the proteins in the blood or serum with an alcohol-acetone mixture gives a green colour as a result of esterification with sulphuric acid and acetic anhydride.

Experimental Procedure

(1) Put 10 mL alcohol-acetone (keep 1: 1 ratio) mixture into the centrifuge tube, add 0.2 mL of blood or serum. Close the mouth of the tube and shake the mixture in a boiling water bath until it boils.

(2) After the tube leaves the boiling water bath, shake it for another 5 minutes. After cooling to room temperature, it is centrifuged for 5 minutes at 1500 rpm.

(3) Transfer the liquid at the top of the tube to another tube. Evaporate the tube containing this liquid until it dries in a boiling water bath.

(4) Cool the tube under tap water. Dissolve the solution completely by adding 2 mL of chloroform to the precipitate.

(5) Add 2 mL of Liebermann reagent (acetic anhydride-sulphuric acid mixture) to the tube and close the tube with parafilm and keep in the dark at room temperature for 10 min.

(6) Measure the absorbance of the solutions at 680 nm.

Tube No	Standard I	Standard II	Cloroform	Liebermann reagent
1	1 mL	-	1 mL	2 mL
2	2 mL	-	-	2 mL
3	-	1.5 mL	0.5 mL	2 mL
4	-	2 mL	-	2 mL
5 (Blank)	-	-	2 mL	2 mL

The cholesterol amount in the tubes prepared from standard I and standard II cholesterol solutions are calculated as mg / dL and plotted against the absorbance values obtained. Dilution factor should be taken as 4.

Solutions:

Stock cholesterol solution (2 mg / mL): 40 mg of cholesterol is dissolved in 5 mL of chloroform and its volume is completed to 20 mL with the addition of chloroform.

Standard I (0.1 mg / mL): Take 1 mL of stock cholesterol solution, complete the volume to 20 mL with chloroform.

Standard II (0.2 mg / mL): Take 2 mL of the stock cholesterol solution and complete the volume to 20 mL with chloroform.

Liebermann reagent (acetic anhydrate-sulfuric acid mixture): Place a beaker containing 60 mL of acetic anhydride in an ice bath. Then, add 10 mL of concentrated sulphuric acid with continuous stirring. Then 30 mL of acetic acid and 0.6 g of anhydrous Na_2SO_4 are added. The reagent can be stored for 1-2 weeks.

4.4.6 Experiments to Identify Ketone Bodies

4.6.1. Identification of Ketone Bodies by Lieben Method

Principle of the experiment: It is based on the principle that acetone forms iodoform with iodine in alkaline medium.

Procedure:

1 mL acetone and 2 mL 2 N NaOH (dropwise) solution are added to a test tube. Then 3 mL of lugol solution is added and mixed. It is observed that a yellow colored precipitate is formed in the tube and the iodoform smell is released.

Solutions:

Lugol solution: 5 g iodine and 10 g KI are dissolved in 100 g distilled water. It is used by diluting 1/5.

2 M NaOH solution: 8 g NaOH is weighed and it is diluted to 100 mL with distilled water.

Acetone

4.6.2. Identification of Ketone Bodies by Legal Method

Principle of the experiment: It is based on the principle that acetone forms cherry red color with sodium nitroprussion in alkaline environment.

Experimental Procedure:

5 ml of acetone, 1 ml of 5% sodium nitroprous solution and 2 ml of 10% NaOH solution are put and mixed in a test tube. It is observed that the mixture in the tube turns red. 1-2 drops of acetic acid are added to the red mixture in the tube; it is observed that the color turns purple.

Solutions:

5% sodium nitroprussium solution: 5 g sodium nitroprussion is weighed and volume is completed to 100 mL with pure water.

10% NaOH solution: 10 g NaOH is weighed and diluted with distilled water to 100 mL.

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