CHAPTER 9

CHOLESTEROL

§ 1. COMPOSITION

455.

	BY WEIGHT		BY VOLUME[1]
Oxygen.....	3.025	100	1.00
Carbon	85.095	2813	36.77
Hydrogen.....	11.880	392.7	63.03

§ 2. PHYSICAL PROPERTIES

456. It is a solid up to a temperature of 137°C[2] where it seemed to me to start melting. When it is liquid, if it is allowed to cool slowly, it crystallizes into radiating flakes. It can also be obtained in the form of very shiny flakes by letting it separate slowly from an alcoholic solution.

457. At a temperature of about 360°C[3], it evaporates under vacuum without decomposition.

458. It is tasteless and odorless or almost odorless.

§ 3. CHEMICAL PROPERTIES THAT ARE OBSERVED WITHOUT THE CHOLESTEROL BEING ALTERED

459. It is insoluble in water.

460. When 100 parts of alcohol with a density of 0.816[4] (g/mL) were brought to the boil, they dissolved 18 parts of cholesterol.

When 100 parts of alcohol with a density of 0.840[5] (g/mL) were brought to the boil, they dissolved 11.24 parts of the same.

On cooling, the alcoholic solutions deposit most of the cholesterol in the form of shiny flakes.

461. Cholesterol exerts no effect on either litmus or hematin. It therefore acts neither as a base nor an acid in contact with colored reagents.

462. When gently heated with lead oxide, no water is liberated.

463. When cholesterol is treated with boiling aqueous potassium hydroxide for at least a hundred hours, it does not show any sign of alteration, as shown by the following experiment. An amount of 1 g of cholesterol and a solution of 1 g of potassium hydroxide in 30 g water are placed in a 2 liter round bottomed flask. This is boiled for twenty-four hours while taking care to replace the water that evaporates. The liquid foams vigorously because of the agitation. When taken off the boil, a few yellow flakes separate out but nearly all the cholesterol retains its crystalline appearance. An extra 4 g of potassium hydroxide is added and boiling is continued for at least six to seven hours per day for two weeks. After this period of time, there is a *partially gelatinous deposit* that is removed by filtration after dilution with water. After concentration, the filtrate sets as a gel as a result of the concentration and the cooling; this gel is a potassium silicate. The silicon originates from the glass of the flask. When the mother liquor of this gel was concentrated and cooled repeatedly until it ceased to become cloudy, it deposited only a few centigrams[6] of yellow colored silica after neutralization with hydrochloric acid. When the filtrate was evaporated to dryness, the resulting residue released into alcohol only traces of a yellow, water-soluble substance and an oily material resulting from the alteration of several atoms of cholesterol by contact with air and heat. What did not dissolve was pure potassium chloride. With respect to the *partially gelatinous deposit*, after having been washed and dried, it turned out to consist of silica that was colored pink by iron oxide, and cholesterol that could be separated by means of boiling alcohol. On cooling, this cholesterol precipitated in the form of glossy, pearly flakes that melt at 137°C[7]. When these flakes are dissolved in alcohol once more, they do not affect litmus or hematin[8] and leave no deposit after burning. These results are in accordance with those of Mr. Powel, but not with the findings of Fourcroy and Mr. Bostock, who regard cholesterol as a saponifiable compound.

§ 4. CHEMICAL PROPERTIES THAT ARE OBSERVED WHEN THE CHOLESTEROL IS ALTERED

Effect of heat and air

464. When an amount of 2 grams of cholesterol is heated in a glass retort, it melts, releases a slight vapor and then starts to boil. The cholesterol turns yellow and later brown. It leaves only a trace of carbon. Almost the entire distillate is liquid and of an oily appearance; it does not turn litmus paper red and it does not contain ammonia[9]. The first distillate is colorless and the last distillate has a reddish yellow color. This product consists of some cholesterol that had not altered and an empyreumatic oil that keeps the cholesterol in the liquid state.

465. When cholesterol is heated sufficiently in contact with air, it catches fire in the same way as wax.

Effect of sulfuric acid at 60°C and in contact with air

466. As soon as 0.2 g of cholesterol flakes in a glass tube with an interior diameter of 0.01 m are brought into contact with 2 grams of sulfuric acid at a temperature of 27 °C, they become an orangey red; the flakes soon fuse together and after two hours, they collect on the surface of the acid as a reddish brown substance with a bituminous appearance. At this stage, sulfur dioxide is given off and can be smelled, and the sulfuric acid becomes a yellowish brown color. The sudden discoloration of cholesterol by sulfuric acid distinguishes it from stearic, palmitic and oleic acids as well as from spermaceti[10]. After contact for seven days, the liquid is dark red and the bituminous substance has been reduced to a black viscous mass. Of all the fatty species, cholesterol produces the most sulfur dioxide without being heated. If the material is heated at 100°C for an hour, sulfur dioxide is released without effervescence, and this is certainly mixed with hydrogen sulfide. Above 100°C, there is effervescence and carbonization of the cholesterol.

Effect of nitric acid

467. When 2 grams of cholesterol are heated with 200 grams of nitric acid with a hydrometer reading of 32[11], nitrous vapors are evolved quite promptly. The cholesterol softens, turns yellow and then becomes a yellow liquid. By operating as described earlier (46), a yellow, astringent and bitter residue results that weighs 1.637 g and can be separated into an *aqueous extract* A and an *alcoholic extract* B.

A. Aqueous extract

468. On evaporation to dryness over a water bath, it leaves a syrupy yellow material with an acid, bitter and astringent taste[12] that forms precipitates with gelatin and lead acetate.

469. The *precipitate* it forms with the latter is deep yellow. When dried and distilled in a tube that had been completely filled with mercury to remove all the air, it yielded a little water and an odorous oil; for every 0.5 g an amount of 43.1 mL gas was formed that consisted of:

Carbon dioxide…….…	36.50
Nitrogen gas……..…	0.90
Methane[13]……..	1.87
Hydrogen gas……...	3.83

470. When the precipitate is distilled in a tube in contact with air, a product results that smells of ammonia.

471. After the precipitate had been mixed with water and decomposed with hydrogen sulfide, a liquor was obtained that after evaporation to dryness left a yellow substance with an acid and slightly astringent taste; its solution hardly caused any gelatin to precipitate.

472. The liquid from which the lead precipitate separated yields a *second yellow precipitate* on evaporation, which has a stronger color than the first.

473. When the liquor from which the second precipitate separated is treated with hydrogen sulfide, filtered and evaporated, it only yields a trace of a yellow, astringent substance which looked to me very much the same as the one that the lead salt had precipitated.

B. Alcoholic extract

474. When evaporated to dryness, it leaves 150 mg of a transparent, yellow material that looks like varnish. This material is soluble in alcohol. When water is added to the alcoholic solution and it is heated, an *orangey resinous substance* separates out that is soluble in aqueous potassium hydroxide, yielding an aqueous solution of a *substance that is less astringent than the material from extract A* (468).

475. It is likely that all the resinous substance would be converted into a soluble substance if the action of the nitric acid had been allowed to continue for sufficiently long.

§ 5. PREPARATION

4.76. If human gallstones[14] are washed in water and then dissolved in boiling alcohol, and the hot alcoholic solution is then filtered, crystallized cholesterol will be obtained when the solution cools down. Then it is only a matter of filtering, washing the filter cake with cold alcohol and drying. If the crystals are colored, they must be dissolved again in boiling alcohol.

§ 6. NOMENCLATURE

477. The name comes from χολή and στερεά[15], meaning "gall" and "solid" respectively.

§ 7. HISTORY

478. Poulletier de la Salle was the first to obtain cholesterol by treating human gallstones with boiling alcohol. Fourcroy likened this substance to a fatty material that he isolated in 1785 from a human liver that had decomposed spontaneously in contact with air and to another fatty material that he isolated in 1786 from cadavers that had been buried in the ground. He regarded these three substances and spermaceti as belonging to the same species of compounds, which he called *adipocere*[16].

In a memorandum that I presented to the Academy on 19 September 1814, I demonstrated that cholesterol is essentially different from spermaceti and the fatty matter from cadavers and I characterized these three substances by the very different way in which each of them reacts with potassium hydroxide (See Book IV, Chapter 4).

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[1] The molecular formula of cholesterol is $C_{27}H_{45}O$. Accordingly, the author is a bit low in oxygen but his carbon to hydrogen ratio of 0.583 is quite close to the actual value of 0.60.

[2] According to the *Handbook of Chemistry & Physics,* the melting point is 148.5°C.

[3] According to the same reference, cholesterol decomposes at 360°C and boils at 233.5°C when the pressure is 0.5 mmHg.

[4] An alcohol density of 0.816 (g/mL) corresponds to an ethyl alcohol content of 92.5 % (w/w).

[5] Similarly, 0.840 (g/mL) corresponds to 83.3 % (w/w).

[6] A *centigram* is one hundredth of a gram or ten milligrams.

[7] See endnote 2.

[8] See also endnote 46 at the end of Chapter 1 of Book II on page 39.

[9] At the time of writing, cholesterol was called 'cholesterine' and supposed to be a vegetable base like morphine. This is why the author looked for ammonia and reports that he did not detect any.

[10] This is the translation of "cétine" which according to M.G. Barruel, (*Traité de Chimie Technique Appliqué aux Arts et à l'Industrie à la Pharmacie et à l'Agriculture*, Volume 5, page 439, 1860), is almost the only constituent of spermaceti.

[11] If a hydrometer reading of 32 corresponds to a density of 1.32 (g/mL), the nitric acid would have a strength of about 50 % by weight.

[12] This is only one of many examples indicating that chemists were not afraid to taste their reaction products. The non-existence of formal safety regulations left them free to do so.

[13] According to Leopold Gmelin, *Handbuch der theoretischen Chemie* (Franz Varrentrapp, 1827, page 256), "gas hydrogène carburé" is one of several names for marsh gas, methane.

[14] During my undergraduate 'organic chemistry' practical, each student had to isolate a compound from a naturally occurring substance. Some students had to prepare caffeine by extracting tea leaves with chloroform, others had to isolate cystine from cow's horn which they had to collect at the local abattoir and there was also the isolation of cholesterol from gall stones, to be collected at the university hospital.

The purification method is as follows (H.A. Boekenoogen, *De Scheikunde der Oliën en Vetten*, A. Oosthoeks Uitgeversmij., Utrecht, 1948): Gall stones are treated in an extraction apparatus with ether until their original structure is completely lost. After evaporation of the ether, the residue is dissolved in alcohol and boiled under reflux for several hours with alcoholic caustic in order to saponify any fat that might be present.

On cooling, cholesterol precipitates as nice flakes that are removed by filtration and recrystallized in alcohol.

[15] The author is extremely correct in using the feminine form of this adjective, thereby respecting the gender of 'gall', which is female in Greek.

[16] No translation is given for this neologism. It has been assembled from *adipose* meaning 'body tissue used for the storage of fat' and *cire* meaning 'wax', which is not that far-fetched since spermaceti is chemically a wax.