Cholesterol from Human Gallstones

In this experiment, cholesterol will be isolated from human gallstones. Cholesterol is an unsaturated alcohol containing 27 carbon atoms. It is a solid (mp = 148-149°C), and it is insoluble in water but soluble in a variety of hot organic solvents.

The gall bladder is attached to the undersurface of the liver just below the rib cage. It retains bile produced by the liver and feeds it into the upper part of the small intestine as needed for digestion. Bile consists primarily of bile acids, which are carboxylic acids closely resembling cholesterol and which aid in the digestion of fats by functioning as emulsifying agents. The gall bladder also harbors free cholesterol. If the concentration of cholesterol in the bile exceeds a certain critical level, it will come out of solution and agglomerate into particles that grow to form gallstones. An amateur geologist given a bottle of gallstones to identify once labeled them a “riverbed conglomerate” -- and indeed they do resemble stones in color, texture, and hardness. They come in a variety of shapes and colors and can be up to an inch in diameter.

As gallstones collect, they irritate the lining of the gall bladder, causing severe pain, nausea, and vomiting. The stones can block the bile duct and at times even lead to fatal complications. Formerly, the only remedy was major surgery. While surgery remains a frequent procedure, gallstones can sometimes be disintegrated in the gall bladder and the entire organ removed through a small incision in the navel. Consequently, it may soon be impossible to obtain whole human gallstones.

In the average human, approximately 200g of cholesterol is concentrated primarily in the spinal cord, brain, and nerve tissue. Insoluble in water and plasma, it is transported in the bloodstream bound to lipoproteins, which are proteins attached to lipids (fats). Recent research has divided these lipoproteins, when centrifuged, into two broad classes—high density (HDL) and low-density (LDL) lipoproteins. A relatively high concentration of HDL bound to cholesterol seems to cause no problems and in fact is beneficial, but a high ratio of LDL-cholesterol leads to the deposition of cholesterol both in the gall bladder (resulting in gallstones) and on the walls of the arteries (causing a plaque that cuts off blood flow and hastens hardening of the arteries or arteriosclerosis).

Mounting evidence points to unsaturated fats such as those found in vegetable oils as favoring the HDL-cholesterol bond, while LDL-cholesterol formation is speeded by saturated fats such as those found in animals. The HDL-cholesterol level goes down with smoking or eating large amounts of sugar. It goes up with regular exercise and with the consumption of moderate amounts of alcohol.

The average American woman at age 75 has a 50% chance of developing gallstones, while for a man of the same age the chance is only half as great. Gallstones and coronary heart disease are also much more common in overweight people. Almost 70% of the women in certain Native American tribes get gallstones before the age of 30, whereas only 10% of black women are afflicted. Swedes and Finns have gallstones more often than Americans; the problem is almost unknown among the Masai people of East Africa.
Experiment

Swirl 0.5-1 g of crushed gallstones in a 25-mL Erlenmeyer flask with about 10 mL of 2-butanone (per gram of gallstone). Heat gently on a hot plate for a few minutes until the solid has disintegrated and the cholesterol has dissolved (a brown residue, bilirubin, will not dissolve). Filter the solution while hot to remove the bilirubin, using a Hirsch funnel and a small filter flask (50-, 75-, or 125-mL). Use some additional hot 2-butanone to rinse the Hirsch funnel. Rinse with an additional 10 mL of methanol. Heat the combined cholesterol/butanone/methanol solution back to boiling, and reduce the total volume to ≤15 mL (best observed if you are working in a small flask). Add hot water to the hot solution to achieve saturation. Cool and filter. Let dry for at least a day before taking final melting point and determining your final % yield.

Lab Report Requirements

• Write up your procedure, with full description of what you did and what you observed.
• Include your percent yield
• Include the melting point for your product cholesterol.
• Given the structure of cholesterol, explain why it is more soluble in organic solvents than in water. Explain why the solubility of free cholesterol in the blood stream is so low.
• Given that cholesterol has 27 carbon atoms, how many hydrogen atoms does it have?