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# Isolation of Cholesterol from Egg Yolk

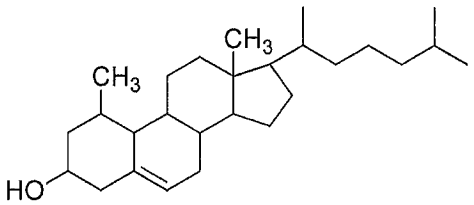
**Source – *adapted from:***

Seager, Spencer and Michael Slabaugh. Safety Scale Laboratory Experiments for Chemistry for Today; Belmont, CA: Brooks/Cole (2005).

*Cholesterol.* (2004). Retrieved February 21, 2007, from http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/C/Cholesterol.html

**Introduction**

Cholesterol is the best-known and most abundant steroid in the body.




The human body contains about 100 g of cholesterol, which is incorporated in the membranes from which cells are constructed and is an indispensable component of them. The insulating layers of myelin wound around neurons are especially rich in cholesterol. It is a precursor for many of the important naturally occurring steroids, including male and female sex hormones, bile salts, and hormones of the adrenal glands. The body also uses cholesterol as a starting material for the synthesis of vitamin D.

One of the major uses of cholesterol is the synthesis of bile salts. These are synthesized in the liver from cholesterol and are secreted in the bile. They are essential for the absorption of fat from the contents of the intestine. A clue to the importance of cholesterol is that most of the bile acids are not lost in the feces but are reabsorbed from the lower intestine and recycled to the liver. There is some loss, however, and to compensate for this and to meet other needs, the liver synthesizes some 1500-2000 mg of new cholesterol each day. It synthesizes cholesterol from products of fat metabolism.

The extreme insolubility of cholesterol in water sometimes causes it to come out of solution. In the bloodstream, this process contributes to the hardening of arteries, and in the gallbladder it leads to the formation of gallstones. Cholesterol isolated from gallstones contains very small amounts of other steroids related to cholesterol.

In this experiment, you will isolate cholesterol from an egg yolk. You will then use the Lieberman-Burchard Test to confirm the presence of cholesterol.

**Safety Information**

2-propanol is flammable; make sure there are no open flames in the laboratory. Glacial acetic acid will vigorously attack tissues; use it with care. Acetic anhydride and conc. sulfuric acid are corrosive and should be used with care.

## Procedure

### *Isolation of cholesterol*

Use a plastic weighing dish and a balance to accurately weigh out a sample of cooked egg yolk with a mass of about 2 g. Record the mass to the nearest 0.001 g in your lab notebook. Put the weighed sample into a 100 mL beaker and add 5 mL of 2-propanol. Stir the mixture with a spatula to break up the egg yolk. Cover the mouth of the beaker with a watch glass to prevent evaporation. Allow the mixture to sit for 10 minutes with occasional swirling while you set up a hot-water bath in a fume hood.

After the mixture has been sitting for 10 minutes, filter it through a dry, fluted filter paper. Collect the filtrate (liquid) in a weighed 50-mL beaker. Be sure to record the original mass of the beaker in your lab notebook (to the nearest 0.001 g). Use a clamp to hold the 50-mL beaker in the hot water bath. Heat the bath to boiling and continue heating until all of the 2-propanol has been evaporated from the beaker. When the evaporation is finished, remove the beaker from the water bath and allow it to cool for 5 minutes. Wipe the outside dry and find the mass of the beaker on the same balance you used before. Record this mass.

Add 20 drops of glacial acetic acid to the residue in the weighed beaker. Swirl the beaker carefully to dissolve the solid residue from the sides and bottom of the beaker. Cover the mouth of the beaker with a watch glass.

### *The Lieberman-Burchard Test for Cholesterol*

Put 4 drops of a 1% cholesterol solution (found on the cart) into a dry 10-cm test tube. Put 4 drops of the cholesterol extract (from your extraction) into a second 10-cm test tube.

Add 4 drops of acetic anhydride and 1 drop of concentrated sulfuric acid ($H_2SO_4$) to each of the test tubes. If cholesterol is present in the test tubes, a characteristic color change occurs as a result of adding these reagents. An initial dark color changes to a blue-green color in 1 to 2 minutes. Note and record the color changes that occur in BOTH test tubes.

## Disposal

Rinse the solid residue from the filter paper with tap water. Put the filter paper into the wastebasket. The contents from the cholesterol test in the test tubes should go into a container labeled "Lieberman-Burchard Test". Excess cholesterol should go into a container labeled "Prepared Cholesterol".

## Postlab

1. Describe the physical appearance of the cholesterol.
2. What mass of cholesterol was isolated.
3. Calculate the percentage of cholesterol isolated from the egg yolk. (What percentage of the mass of the egg yolk was due to cholesterol?)
4. What did the Lieberman-Burchard test tell you? Describe what you saw.

Be sure to include the discussion section in your lab notebook.