Biology and Biochemistry Department
BIOC311
Biochemistry Lab

Experiment #13

Title: (A)Precipitation of Cholesterol from Brain.
(B) Estimation of Cholesterol Amount: The Lieberman Burchard Reaction.

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Objective:

(A) The objective of this experiment is to determine the amount of cholesterol present in the brain and compare it to the theoretical amount.

(B) The objective of this experiment is to use the Lieberman Burchard reaction to detect the presence of cholesterol in the brain. (green-blue color must be detected)

Introduction:

(A) Every cell's plasma membrane contains unsterilized cholesterol as a structural component. This membrane evolved to perform a secondary, highly specialized role in the central nervous system as the primary structural component of compact myelin throughout development. As a result, the central nervous system has the highest mean unsaturated cholesterol content of any human tissue (23 mg/g). While accounting for just 2.1 percent of total body weight, the central nervous system comprises 23 percent of the overall sterol pool. The majority of central nervous system development and differentiation takes place in the first few weeks or years following birth in all animals, and the cholesterol necessary for this growth appears to be created entirely from scratch.. (1)

(B) In the human body, cholesterol is required for the production of bile acid and steroid hormones. Excessively high levels, on the other hand, are generally linked to heart problems. As a result, it's critical to keep track of excessive quantities of it in human diet, necessitating the development of analytical procedures to identify it. In this work, the

Liebermann–Burchard reaction was investigated in order to establish a simple, reliable, and robust quantitative colorimetric procedure for assaying cholesterol as a feasible alternative to chromatographic methods. (2)

Materials:

(A)

- Brains
- Acetone
- Blender
- Hot Plates
- Boiling Water Bath
- Boiling Chips
- Centrifuge Tubes
- Ethanol

(B)

- Cholesterol from (13A)
- Alcohol: Acetone mixture
- Chloroform

- Acidic anhydride sulfuric acid
- Cholesterol stock

Methods: (A)

1. 40 ml of acetone was added to 10 g of the brain and was blended for 1 min.
2. The blender was rinsed out using 10 ml acetone and was blended again for another 1 min.
3. The homogenate was stirred for 10 minutes and the suspension centrifuged at 2000 rpm for 5 minutes.
4. In a small clean beaker, a few drops of boiling chip was added and a beaker was weighed.
5. The supernatant was placed in the weighed beaker, and the acetone was removed by heating the beaker on a hot plate while occasionally stirring.
6. The beaker was cooled in ice water and the crude cholesterol collected.
7. The amount of cholesterol was determined by weighing the cholesterol with the beaker.

(B)

1. Different concentrations of cholesterol was prepared using the stock solution.
2. Chloroform in a total volume of 2 ml per dilution and the blank was 2 ml of chloroform.
3. 2 ml of your isolated cholesterol samples was prepared by mixing known volume

with chloroform.

4. 2 ml of the acetic anhydride-sulfuric acid mixture was added to all tubes and were mixed thoroughly.
5. The Tubes was left in the dark at room temperature for 3 minutes and the Extinction was read at 680 mm.

Data and results:

A	
Weight of brain	70.1g
Total volume of acetone including rinsing	560.8ml
Weight of beaker &boiling chips	99.7g
Weight of beaker with cholesterol	102.0g
Weight of total cholesterol obtained	2.3g
Theoretical weight of cholesterol in cow brain per 70.1g	2.1731g (3)

Table (1) data obtained from extracting cholesterol from a cow's brain.

B	
Tube	***absorbance***
1	0.682
2	0.282
3	0.092
Isolated cholesterol	1.432

Table (2) tube number and its absorbance after the addition of ninhydrine.

*****Conc of unknown cholesterol = 0.39 g/ml**

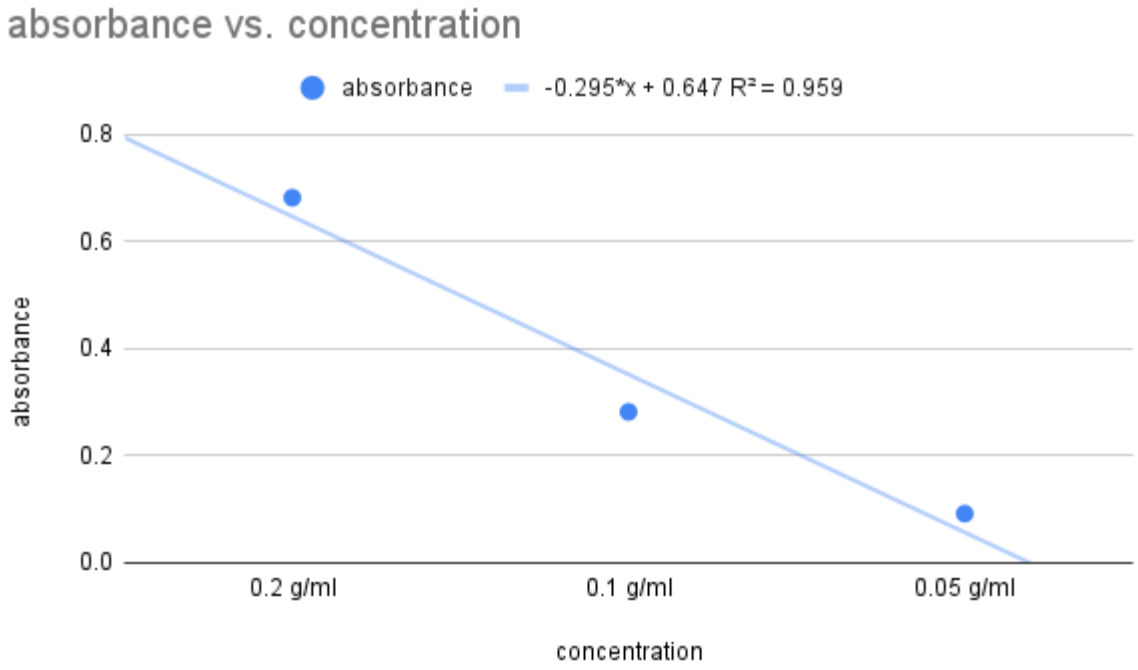

Chart (1) absorbance vs concentration of a known sample of hemoglobin.

Discussion :

(A)

In part A, a frozen cow brain was blended with acetone for multiple blends. The last blend was a wash to ensure the complete blending of the acetone. Acetone is used because of its capability to dissolve organic compounds. Onward, the total cholesterol obtained was 2.3g. This may seem

higher than the theoretical, but its unsure due to the differences in sources. The concentration of the cholesterol obtained in this brain sample is unknown yet.

(B)

In part B, the concentration of a known sample of cholesterol was found through its absorbance. Then, an assay curve was made in order to identify the concentration of the unknown sample of cholesterol. The cholesterol got its color from a combination of ninhydrin and chloroform. This should result in an array of blue-green shades that indicate the presence of cholesterol. Chart 1 shows the decrease in concentration, with the increase of absorbance. Due to the trendline on the graph, we were able to obtain the concentration from part A. This concentration is 0.39g/ml.

Conclusion :

In conclusion, the lieberman burchard method is efficient for the detection of the concentration of cholesterol. But, the method of extraction is really efficient. This is especially because of multiple systematic errors that could occur.

References:

1. Dietschy, J. M., & Turley, S. D. (2004, May 16). *Thematic review series: Brain lipids. cholesterol metabolism in the central nervous system during early development and in the mature animal*. Journal of Lipid Research. Retrieved November 29, 2021, from https://www.sciencedirect.com/science/article/pii/S0022227520313006

2. Li, L. H., Dutkiewicz, E. P., Huang, Y. C., Zhou, H. B., & Hsu, C. C. (2019). Analytical methods for cholesterol quantification. *Journal of food and drug analysis*, *27*(2), 375-386.

3. Leaf Group. (n.d.). *Nutrition value of Beef Brain*. LIVESTRONG.COM. Retrieved November 29, 2021, from https://www.livestrong.com/article/360367-nutrition-value-of-beef-brain/ .

Appendix :

(A)

70.1 g * 10ml acetone / 40g =280.4ml acetone

(B)

total volume of mixture chloroform with cholesterol = 1ml

C1V1=C2V2

Tube 1 =0.4* 2 = M2 * 4 = 0.2 g/ml

Conc. of unknown cholesterol

Y= 3.9429X- 0.108

1.432 = 3.9429X-0.108

X= 0.39 g/ml