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Diploma in Pharmacy 2nd Year
Biochemistry & Clinical Pathology
Experiment
To determine the cholesterol in blood/serum.

Aim:

To determine the cholesterol in blood/serum.

Reference :

‘ Dr. Gupta G.D. , Dr. Sharma Shailesh, Kaur Manpreet, “Practical Manual of Biochemistry & Clinical Pathology” Published by Nirali Prakashan, Page no 44 – 48

Materials Required

Absolute alcohol, Diethyl ether, Chloroform, Acetic anhydride, Standard solution of cholesterol (100 mg%), test tube and stir rod.

Theory :

The blood contains both esters and free cholesterol Normally, the ester fraction forms 70% of the total cholesterol while free cholesterol forms roughly 30% An alcohol-ether mixture is used to extract the cholesterol and cholesterol esters from the serum (Alcohol precipitates the proteins or either solubilises the cholesterol part). The contents are centrifuged. The protein free extract is dried using evaporation. Using the Liebermann-Burchard procedure, the cholesterol residue is dissolved in chloroform and colorimetrically quantified.

Reagent Preparation

100 mg of cholesterol should be dissolved in some amount of alcohol. It should be slightly warmed (in water bath), if needed. The volume should be maintained to 100 ml. with alcohol.

Procedure

1) Test:

i. 8mL of alcohol and 2ml. of ether should be taken in a centrifuge tube and then mixed.
ii. 0.2mL of blood should be added and mixed. It should be kept in slanting position for half an hour and centrifuged
iii. The supernatant should be collected (which contains cholesterol) in another tube. This test tube should be kept in boiling water for the evaporation of solvent and the residue, ie, leaving behind a residue of cholesterol that adheres to the bottom of the flask.
iv. The best quality chloroform, acetic anhydride, and sulphuric acid should be used. Chloroform needs to be extremely anhydrous. The colour produced by regular chloroform or outdated stock will be weak and unpredictable.

2) Standard:

i. 0.2, 0.4, 0.6 and 0.8ml. of standard cholesterol solution should be added to the labelled tubes, 1.e., S1, S2, S3, and S4
ii. All test tubes should be stored in a hot water bath to allow the solvent to evaporate and leave any residue behind.
iii. The blank test tube should be cleaned and dried.
iv. The following reagents should now be added in test, different standard, and blank.

Reagent	Blank	Standard				Test
		S_1	S_2	S_3	S_4	
Standard cholesterol solution	–	0.2mL	0.4mL	0.6mL	0.8mL	Test sample
$CHCl_3$	5mL	5mL	5mL	5mL	5mL	5mL
Acetic anhydride	2mL	2mL	2mL	2mL	2mL	2mL
Concentrated H_2SO_4	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL

v. It should be mixed well and kept in dark for 10 minutes.
vi. The optical density should be read at 610nm.

Calculations

Optical density of test =

1) Optical density of standard S_1 = concentration = 0.02 mg
2) Optical density of standard S_1 = concentration = 0.04 mg
3) Optical density of standard S_1 = concentration = 006 mg
4) Optical density of standard S, concenuation = 0.08 mg

$$\text{The concentration of cholesterol} = \frac{\text{Optical density of test}}{\text{Optical density of std.}} \times \frac{\text{Conc of std.}}{\text{Vol. of blood/serum used}} \times 100$$

Interpretation

1) Normal serum cholesterol level is 150-250 mg/dl.
2) The normal level of serum cholesterol is 150-250 mg/dl.
3) Following conditions are caused due to hypercholesterolemia:
   i) Diabetes mellitus
   ii) Obstructive jaundice
   iii) Nephrotic syndrome.
   iv) Cirrhosis of liver
   v) Hypoparathyroidism.
   vi) Xanthomatosis.
4) Following conditions are caused due to hypocholesterolemia:
   i) Hyperthyroidism
   ii) Pernicious anaemia
   iii) Haemolytic anaemia
   iv) Malabsorption syndrome

Result :

Cholesterol concentration in blood /serum was determined.