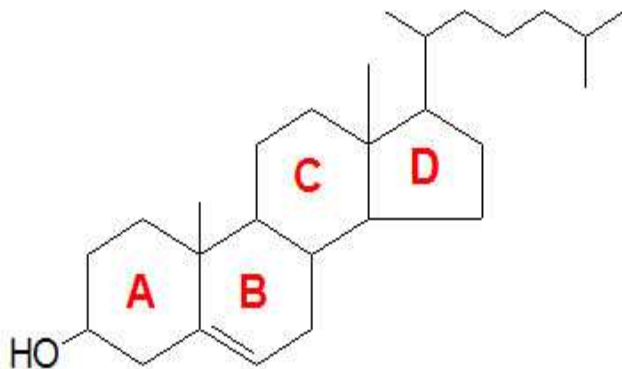


Assay for Total Cholesterol

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Biochemistry Lab

Rationale

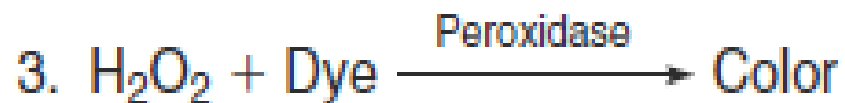
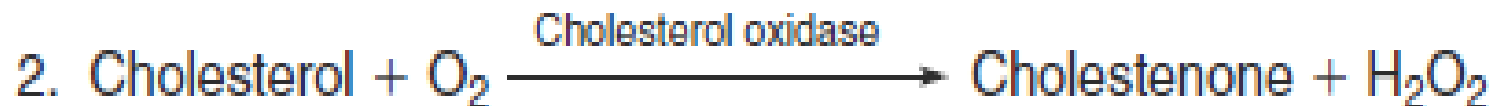
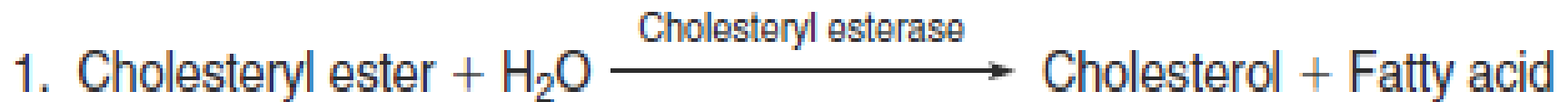
- Cholesterol is steroid alcohol synthesized in hepatocytes and is necessary for the production of bile acids, steroids, and cellular membranes.
- Total cholesterol comprises all of the cholesterol found in various lipoproteins (major component of LDLs and a minority component of HDLs and VLDLs)
- Total cholesterol has been used for aids in the detection of many conditions bound to metabolic disorders.

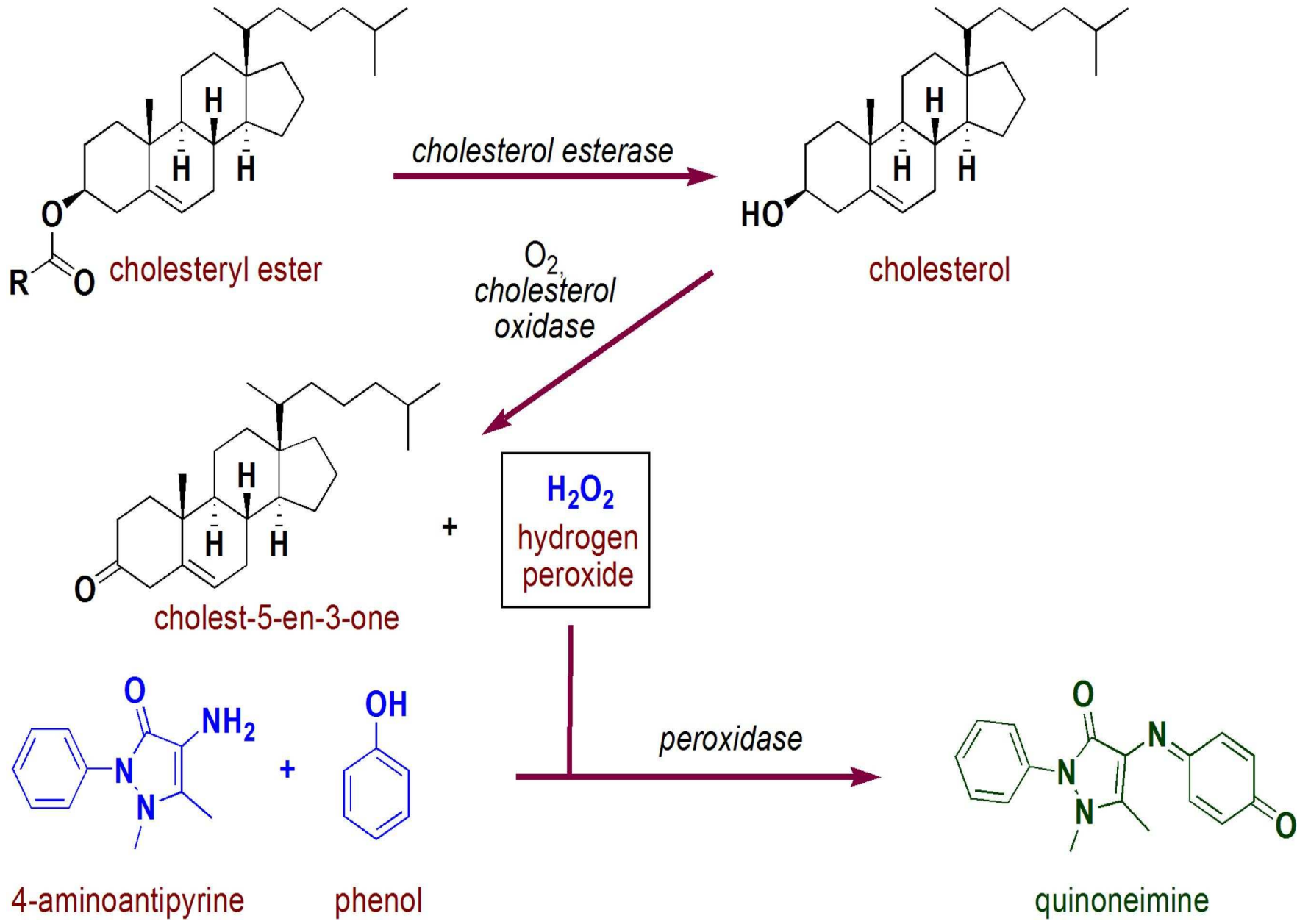
- Certain diseases are known causes of hypercholesterolemia
 - Cholestasis
 - Hypothyroidism
 - Diabetes
 - Nephrotic syndrome
- A high-cholesterol diet is another important factor that must be considered.
- Decreased cholesterol values found in hepatic atrophy or hypoplasia, hyperthyroidism

Total Cholesterol (TC) Measurement

Specimen : Serum/ Plasma

- Measurement of total cholesterol involves the use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CO) and peroxidase (POD) in a series of reactions in which cholesteryl esters are hydrolyzed, the 3-OH group of cholesterol is oxidized and H_2O_2 is quantified.





Interferences

- Reducing substances such as ascorbic acid and bilirubin can interfere with measurements by consuming H_2O_2 .
- Lipemic samples generate turbidity of sample which leads to falsely elevated values (chylomicrons gives the appearance : Postprandial)



Materials

- Spectrophotometer
- Micropipettes
- Pipette tips (Small & Large)
- Test tubes with rack
- Cholesterol Monoreagent
- Cholesterol Standard Solution
- Test Sample
- Control Solutions

Procedure

| | Blank | Standard | Sample |
|-------------|--------|----------|--------|
| Monoreagent | 1000ul | 1000ul | 1000ul |
| Standard | ----- | 10ul | ----- |
| Sample | ----- | ----- | 10ul |

Mix and measure the absorbance at 500 ± 10 nm after incubating at $+37^{\circ}\text{C}$ for 5 min or 10 min. at $+25^{\circ}\text{C}$.

Calculation :

$$C_{\text{test}} = \frac{A_{\text{test}} \times C_{\text{std}}}{A_{\text{std}}}$$

$C_{\text{std}} = 200\text{mg/dl}$

| | | | |
|------------------------|---------|--------|-------------|
| Normal values (mg/dl) | Dog | Cat | Herbivorous |
| | 126-144 | 90-108 | 36-54 |