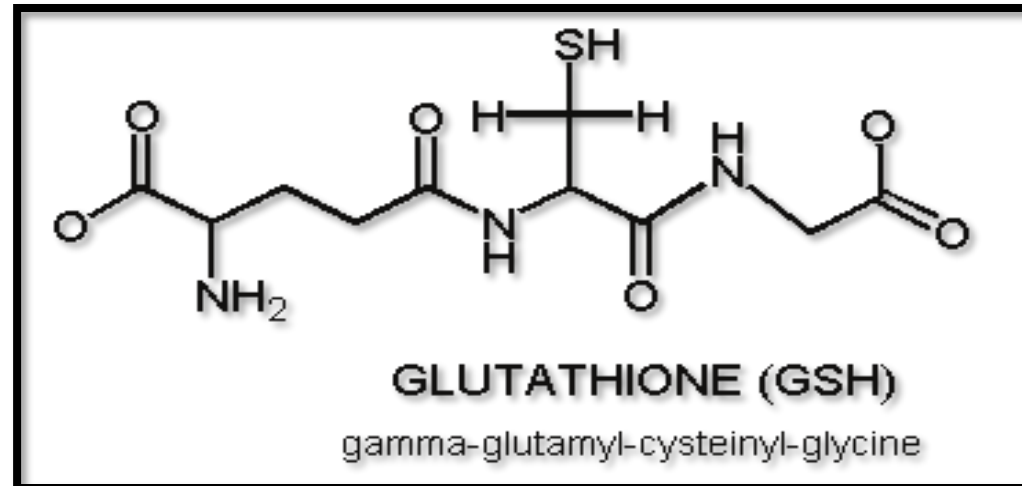




BCH 447

## Estimation of Glutathione in Plasma



## - Objectives:

- ✓ To draw the standard curve of glutathione by given known amount of glutathione assay procedure using spectrophotometric technique.
- ✓ To estimate the amount of glutathione in plasma sample.

# Glutathione

- It occurs naturally as a **tripeptid** (gamma-**glutamylcysteinylglycine** )
- it has two form : Reduced form(GSH) and Oxidized form (GSSG)
- It has nucleophilic and reducing properties, those properties **due to the presence of Sulfahydryl group or thiol group (-SH).**

**\*\*Nucleophelic**, means GSH will loss the H ion then become nucleophilic (contain negative charge) can react with the compound which carrying positive charge.

**\*\*reducing** , means it will give the other protein the H ion to maintain them in reducing form.

# Functions of Glutathione

- It acts as anti-oxidant , blood booster , and cell detoxifier in the body.

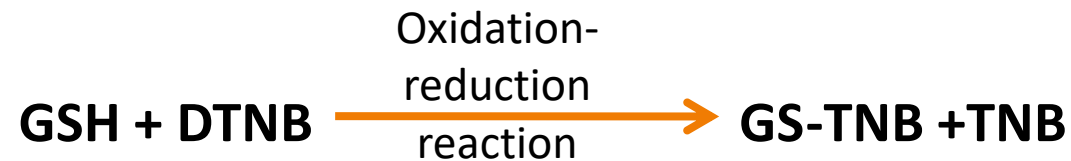
## GSH is crucial to a variety of life processes, including

<b>Amino acid transport across membrane</b>	<b>Maintenance of the SH level of proteins</b>	<b>detoxification of xenobiotics such as drugs</b>	<b>Removal of hydroperoxide and free radicals.</b>	<b>Coenzyme with glutathione peroxidase, glutathione S-transferase and thiol transferase.</b>
---	--	--	--	---

- Physiological values of intracellular GSH generally range **from 1 to 10 mM**.
- Glutathione deficiencies have been linked to many forms of **cancer**.

# Assay Principle

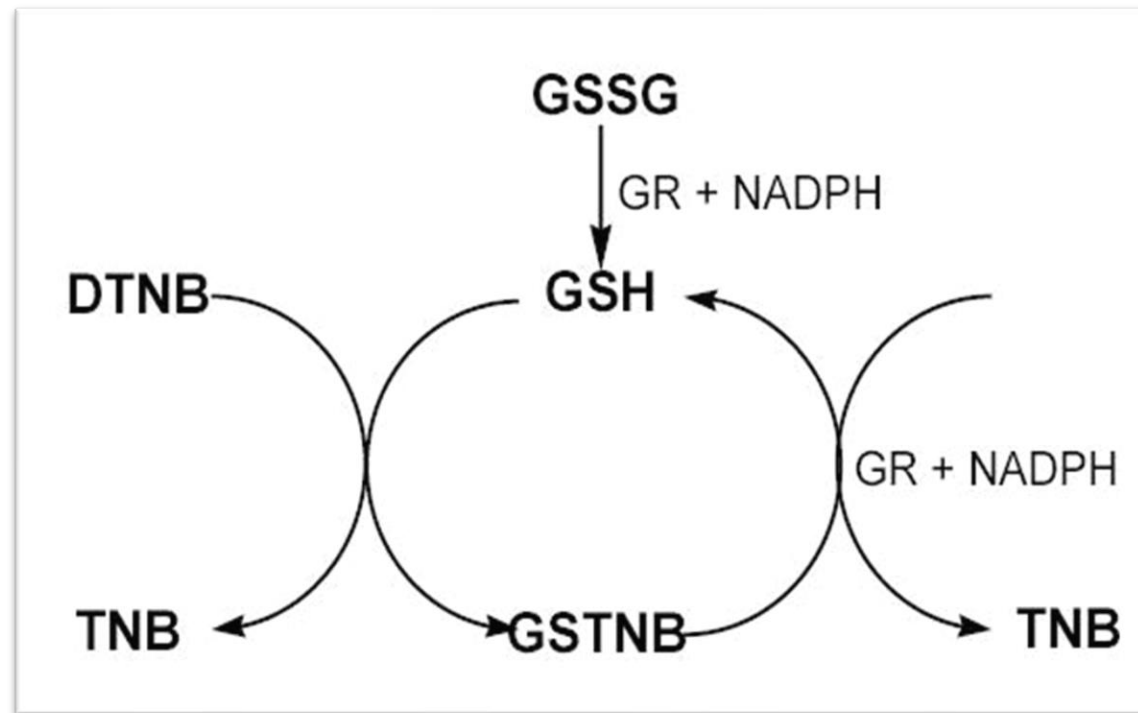
- The principle of the assay is based on **the oxidation of the reduced form of glutathione (GSH)** by the aromatic disulphide compound [5,5-dithiobis-2-nitrobenzoic acid (DTNB)] to form GS-TNB and the aromatic thiol, 5thio-2nitrobenzoic acid (TNB).



- Yellow color is formed.** It can be measured **at 412nm** and is proportional to the amount of glutathione present in the sample.

**Note** : This method is also useful to determine the GSSG.

First , GSSG will be converted to GSH by glutathione reductase (GR) and NADPH assay then it will react with DTNB reagent



## - Method:

1- Prepare serial GSH concentration, as the following table:

Tube No.	GSH stock Solution (ml)	Phosphate Solution (ml)	Total Volume (ml)	GSH Conc (mg/dl)
Blank	0	3	3	0
1	0.6	2.4	3	2
2	1.2	1.8	3	4
3	1.8	1.2	3	6
4	2.4	0.6	3	8
5	3	0	3	10

**2- For standard curve (Tubes from blank to 5)**

In a separate test tube, take 0.5 ml of solution+ 2ml of Phosphate Solution + 0.25ml DTNB+ 0.25ml H<sub>2</sub>O.

**3- For Sample:** Take 0.3 ml of sample +2ml of Phosphate Solution + 0.25ml DTNB+ 0.45ml H<sub>2</sub>O.

\*\*Incubate all tubes for 10min at 37°C → Read absorbance at 412 nm \*\*

## - Results:

Tube No.	GSH concentration (µg/ml)	Absorbance At 412 nm
1	20	
2	40	
3	60	
4	80	
5	100	
Blank	0	
Sample	From Sandard Curve	

- **How to convert concentration unit from mg/dl to µg/ml ?**
- 2 mg/dl to µg/ml  $\longrightarrow 2 \times 1000/100 = 20 \mu\text{g/ml}$



- **Calculations:**

- Plot the standard curve and determine glutathione concentration from the graph expressed as  $\mu\text{g/ml}$ .
- Calculate the glutathione concentration in Total volume of extracted plasma (= 3 ml).

• Example:

0.3 ml of sample  $\rightarrow$  0.2  $\mu\text{g}$

3 ml of plasma  $\rightarrow$  ?

- Calculate glutathione concentration in mg by converting  $\mu\text{g}$  to mg.

Normal range of glutathione concentration = 3.344-4.84 mg