EXPERIMENT NO: 5

THE EVALUATION OF LEAD EXPOSURE

DETERMINATION OF δ-AMINOLEVULINIC ACID (ALA) IN URINE

A) General Information:

The most important biological effect of lead is on the hematopoietic system. The lead affects hem biosynthesis: it inhibits the ALA-dehydraz and ferrochelatase enzymes in this system**.**

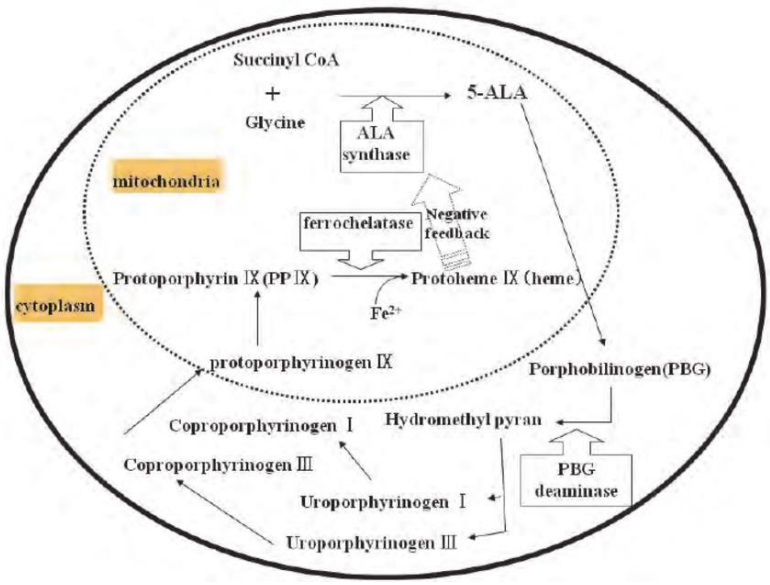


Figure 1 shows the control of heme synthesis in the cell. The effect of free hem pool on ALA-synthetase and microsomal enzymes is important. The regulator of the total hem components in the liver is the free hem pool. Heme combines with globin to form hemoglobin. Hemoglobin contains Fe + 2 and is responsible for oxygen transport to tissues. Heme is also found in the structure of Cytochrome P-450. The mixed function oxidase system, which is the most important system that provides the metabolism of the drugs and other chemicals taken into the organism by oxidation, is essential for the formation of Cytochrome P-450 (CYP 450). It is thought that lead intoxication can suppress microsomal CYP 450 synthesis and change drug metabolism.

Figure 2 shows the effects of lead on both biosynthesis. Enzymes inhibited by this system as a result of exposure to lead:

• ALA-D

• Ferrochelatase enzymes.

As a result of the inhibition of ALA-dehydratase, δ -ALA cannot turn into PBG. With the inhibition of ferrochelatase, The Fe+ 2 ion in the cytoplasm cannot enter the mitochondria and as a result hem does not occur. Due to the decrease in the amount of hem, the negative feedback effect on the δ -ALA synthetase, the rate-limiting enzyme of hem, is suppressed, and the δ-ALA synthetase activity and δ-ALA amount produced are increased.

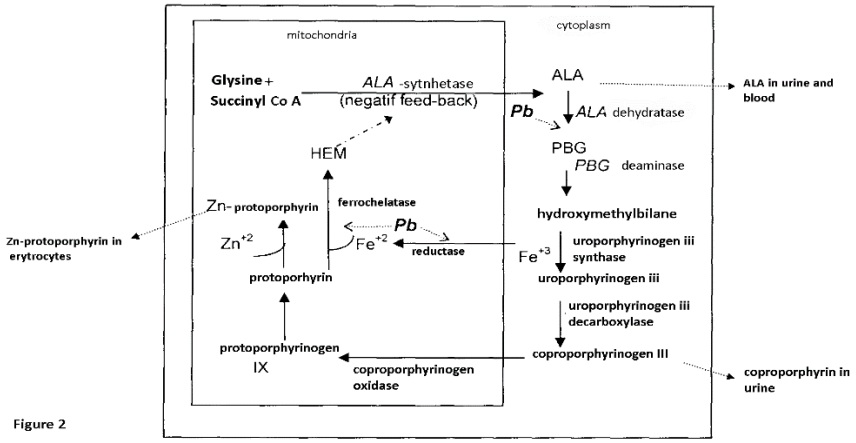


Figure 2

δ-Aminolevulinic acid affects neuromuscular functions; causes neurological and behavioral disorders. In addition to this neurotoxic effect, ALA also has prooxidant properties. Increased ALA due to lead toxicity may cause lipid peroxidation.

Urine ALA level;

• **Normal** up to 6 mg / L,

• 6-20 mg / L is **acceptable**,

• **increased** to 20-40 mg / L,

• Over 40 mg / L is interpreted as **hazardous**.

B) Principle of δ-Aminolevulinic Acid Assay Experiment in Urine

The solutions used in the experiment:

• Acetate buffer: glacial acetic acid, sodium acetate trihydrate and distilled water.

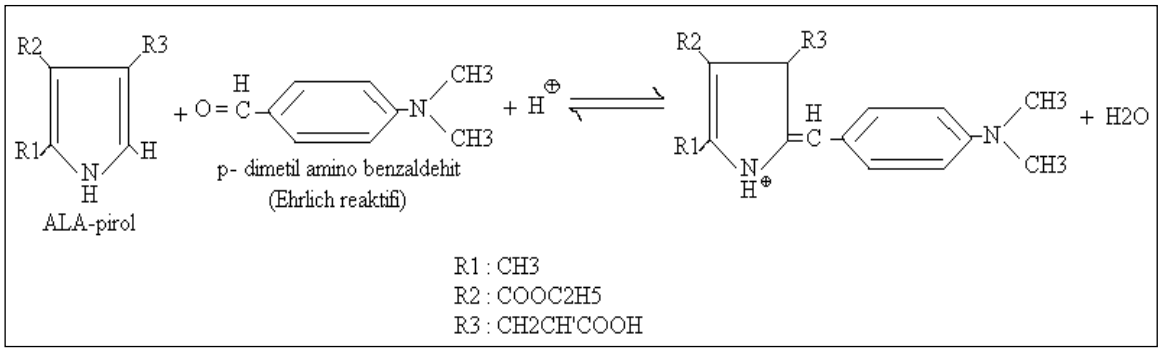
• Ethyl acetoacetate

• Ethyl acetate

• Modified Ehrlich reagent: Consists of glacial acetic acid, p-dimethyl amino benzaldehyde, perchloric acid and distilled water.

Principle of the experiment:

Principle of the experiment: Determination of the amount of δ-ALA in urine is a good bioindicator for lead exposure. In this experiment; ALA-pyrrole condensation occurs with the aid of ethyl acetoacetate. The resulting complex is extracted with ethyl acetate aqueous solution. The ALA-pyrrole complex is treated with Ehrlich reagent as colorimetric (cherry-red color).



Calibration curve using standard ALA solutions

For this purpose, a series solution is prepared such that 1 mg / L, 3 mg / L, 5 mg / L, 10 mg / L, 15 mg / L is prepared from the stock solution bir-ALA.HCl. The absorbance of these solutions is read by the experimental procedure. The ALA calibration curve is drawn from the obtained concentration-absorbance values and the calibration equation is extracted.

Experimental Procedure:

1. Take 2 test tubes and add 1 mL of urine to each.

2. Add 1 mL of acetate buffer (pH: 4.6) to each 2 tubes.

3**. Establishment of ALA-pyrrole complex:**

Add 0.2 mL of ethyl acetoacetate to one of the tubes and vortex for approximately 5 seconds

(Ethyl acetoacetate is not added to the tube to be used as blank).

4. Heating:

Both tubes are closed and placed in a water bath for 10 minutes (at the 10th minute of heating, the ALA-pyrrole concentration is maximum).

5. Cooling:

At the end of the 10th minute, the tubes are removed from the water bath and allowed to cool.

6. Extraction of ALA-pyrrole:

Add 3 mL of ethyl acetate to both tubes and vortex the tubes to extract the ALA-pyrrole.

7. Centrifuge:

Tubes are centrifuged for 3 minutes (at 2,000 rpm).

8. Pipette 2 mL of supernatant (ethyl acetate phase) and transfer to another tube.

9. Addition of Ehrlich reagent:

2 mL of Ehrlich reagent is added to the tubes and vortexed.

10. Tubes are allowed to stand for 10 minutes**.**

11. Absorbance measurement:

Measure the absorbance of the resulting colored solution at 553nm.

Calculations and evaluation:

For the calculation of ALA concentration in urine;

Absorbance (A) measured by the addition of ethyl acetoacetate,

Absorbance (B) measured without addition of ethyl acetoacetate is removed.

$A_{abs.} - B_{abs.} = ALA_{abs.}$

The obtained ALA concentration of urine ALA absorbance value is replaced in the standard calibration equation and the concentration of urine ALA is calculated and the exposure is evaluated.