CHAPTER I

INTRODUCTION

Lead (Pb) has been mined and worked by men for millenniums. It is one of the most useful metals (Chisolm, 1971). The main use of Pb is in the manufacture of storage batteries , smelter works , foundries , vehicle radiator repair, and as alloys combined with various metals that provide certain qualities suitable for the particular use (Winship , 1989 and Phannee Pidetcha *et al.* , 1990). Afterwards Pb is also used extensively in the chemical industries , paint , insectiside , ceramic and cosmetic products (Winship, 1989).

1.1 Pb Sources , Absorption and Excretion

Although Pb is utilized for men , its inappropriate use has , however , resulted in outbreaks of Pb poisoning in humans from time to time since antiquity (Chisolm, 1971). Pb is toxic to most living organisms at high exposures and there is no demonstrated biological need (Goyer, 1991). It is widely distributed in a variety of minerals and is also a constituent of air, water, and the biosphere. Human beings ingest a certain amount of Pb in food , water and from the air (Naovarat Suwanabun, 1993). The principle

route of exposure is food , but it is usually, environmental and presumably controllable sources that produce excess exposure and toxic effects. These sources include Pb-based indoor paint in old dwellings , Pb in air from combustion of Pb-containing auto exhausts or industrial emissions , hand-to-mouth activities of young children living in polluted environments and less commonly , Pb dust brought home by industrial workers on their clothes and shoes , and Pb-glazed earthenware (Goyer, 1991).

The major routes of absorption of Pb are from the gastrointestinal tract and the respiratory system. About 5-10 % of Pb ingested in food is absorbed , mainly in the small intestine ; some of the Pb absorbed undergoes enterohepatic recycling. Children absorb a substantially greater proportion, about 50 % of dietary Pb , than adults. Inorganic Pb is not absorbed through intact skin but organic Pb compounds, such as tetraethyl Pb may be absorbed rapidly. Fume of organic Pb compounds are highly toxic, it can be absorbed from all areas of the respiratory tract, including the nasal passages (Winship, 1989).

Pb contamination in Normal conditions are estimated to be 100-300 mg of Pb per kg of food, 100 mg per liter of water and 2.5 mg per cubic meter of air. By contrast, large urban areas have annual average Pb concentration of 1-3 mg per cubic meter in peak traffic periods (Naovarat Suwanabun, 1993).

In human, urinary excretion is the main route for Pb disposal, accounting for 75-80 %, a lesser amount, about 15% appearing in gastrointestinal secretion. Other routes such as hair, nails and sweat account for less than 8 % (Kehae, 1961). The concentration of Pb in urine is directly proportional to that in plasma , but since most of the Pb is in the erythrocytes , very little is filtered. The elimination of Pb is slow. The half-life of Pb in blood is variously stated to be about one month to 70 days (Klaassen, 1980). Urinary concentrations above 15 mg per liter in adults and 0.08 mg (80 μg) per liter in children are considered to be toxic level (Berman, 1966).

1.2 Clinical Manifestations of Pb Poisoning

1.2.1 Acute Exposure

Acute Pb poisoning occurs from the accidental ingestion of acid-soluble Pb compounds (Klaassen, 1980). The fatal dose of absorbed Pb has been estimated as 500 mg. Symptoms are an intense thirst , a metallic taste , followed by nausea, vomiting and a burning abdominal pain ; there may be diarrhoea or constipation. Acute central nervous symptoms , which include paraesthesia , pain and muscle weakness , develop. An acute haemolytic crisis is seen, resulting in anemia and haemoglobinuria. Severe renal damage follows , with shock and coma ; death may occur within

1-2 days (Winship, 1989).

1.2.2 Chronic Exposure

Chronic poisoning is usually due to the accumulation of small quantities of Pb in the body by inhalation, ingestion or skin absorption. The symptoms are mainly gastrointestinal, neuromuscular, affect the central nervous system, renal effect and haematological effect (Winship, 1989).

Chronic exposure leads to renal damage and can also interfere with fertility and cause female menstrual disturbances. The most severe clinical form of Pb poisoning is brain damage (Naovarat Suwanabun, 1993).

1.3 Pb Toxicity on Heme Synthesis and Blood Pb Concentration

Since hematological effect is the most obvious symptom in Pb toxicity , it is of interest to mention here , the mechanism of this metal on heme synthesis.

Heme is the iron - containing constituent that combines with protein to form hemoglobin, the oxygen-carrying pigment of the red blood cells. Heme is also an essential constituent of the other respiratory pigments, the cytochromes, which play key roles in energy metabolism (Chisolm, 1971). Biosynthesis of heme is shown in Figure 1. Lead has been shown to inhibit heme synthesis at several sites , the enzymes concerned being heme synthetase ,

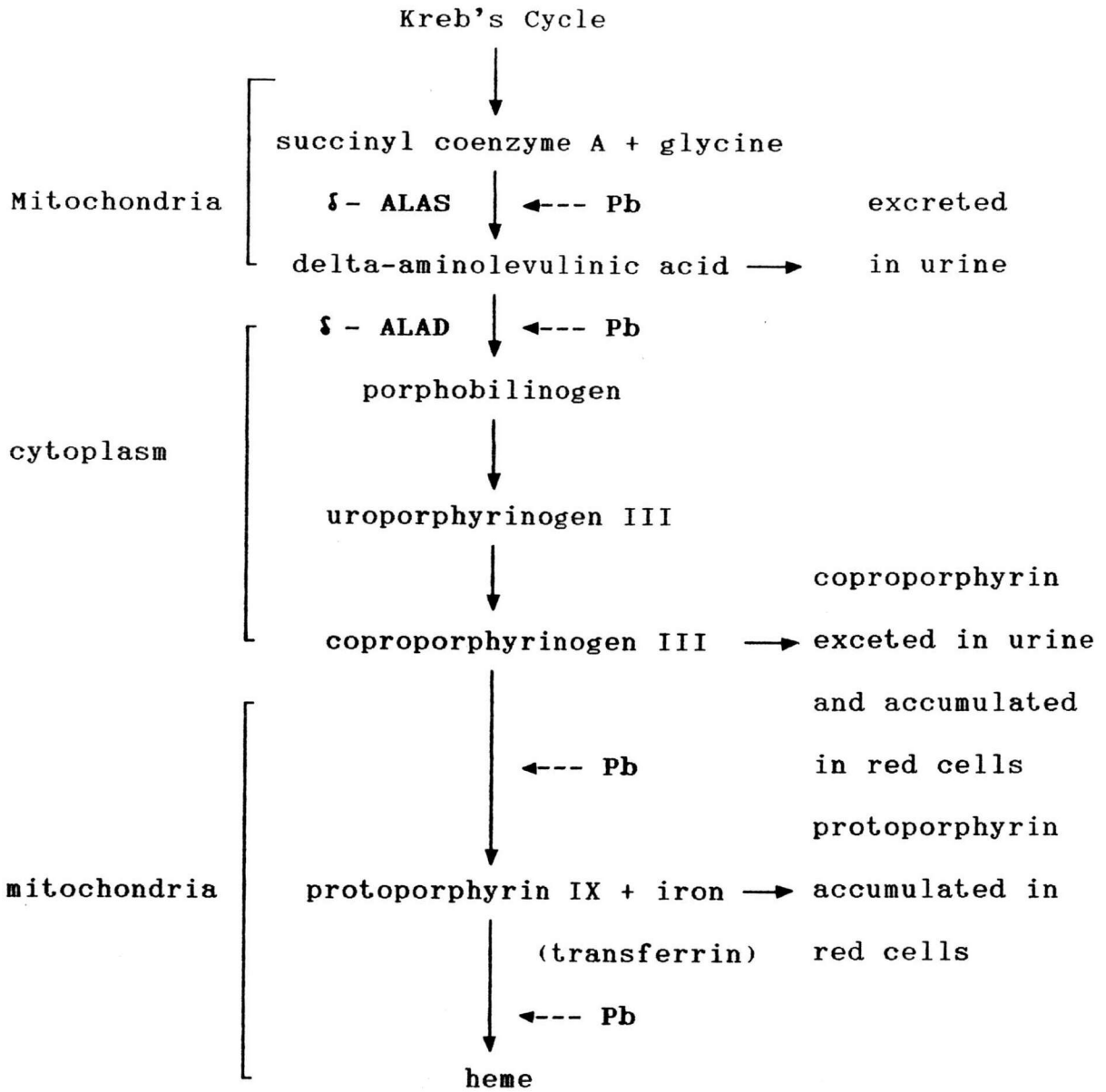

Figure 1 Biosynthesis of heme, a constituent of hemoglobin, is inhibited by lead, resulting in accumulation of intermediates in the synthetic pathway, the first and the last two take place in mitochondria, the another elsewhere in the cell cytoplasm.

(◀---) indicates steps which lead inhibits.

delta - aminolevulinic acid synthetase (δ - ALAS), delta-aminolevulenic acid dehydratase (δ - ALAD) and ferrochelatase (Chisolm, 1971; Nakao, 1968 and Rogan, 1986). These enzymes are sulfhydryl-dependent for activity , and Pb exerts its effect by blocking them.

Inhibition of Lead on activities of δ - ALAS, δ - ALAD and ferrochelatase (Chisolm, 1971; Nakao, 1968 and Rogan, 1986) result in elevation of urinary coproporphyrin (CP) and accumulation of protoporphyrin in the erythrocytes (EP) (Mahaffey, 1986 and Rogan, 1986).

Blood Pb concentration is often used to assess the level of Pb exposure. Criteria point of blood Pb level as an indication for use to determine toxicity is dependent on individuals (Baker, 1979). The minimum blood Pb level at which the effect is likely to be observed are shown in Table 1. These effects involve several organ systems and biochemical activities. (Goyer, 1991)

However haematological toxicity in terms of impairment of heme biosynthesis and acceleration of red cell destruction thereby leading to anemia is associated with blood Pb concentration (Baker, 1979) : values above 300 μg per liter (1.5 μM) would now be regarded as high (Simons, 1993). The study involving red cell enzyme activity of asymtomatic Pb exposed workers and *in vitro* assay of red cell enzymes have shown that the cytoplasmic delta-aminolevulinic acid dehydratase (δ- ALAD) is initially

Table 1 Lowest observed effect levels for induced health effects (Goyer, 1991)

Effects	Blood Pb concentration (µg/l)	
	Children	Adults
heme effects		
anemia	800-1,000	800-1,000
U - ALA	400	400
EPP	150	150
ALAD inhibition	100	<100
neuro effects		
Encephalopathy	800-1,000	1,000-1,200
I.Q. deficits	<300	-
Peripheral neurepathy	400	400
renal effects		
Acute nephropathy	800-1,000	-
Chronic nephropathy	-	600
Vit D-metabolism	<300	-

U-ALA = Urinary aminlevulinic acid

EPP = erythrocyte protoporphyrin

ALAD = aminolevulinic acid dehydratase

inhibited by blood Pb concentrations of 100-200 μg per liter and is more or less completely inhibited at Pb concentrations of 700-900 μg per liter (Lolin and O'Gorman, 1986 ; Phannee Pidetcha *et al.*, 1990). Another enzyme that is inhibited is the ferrochelatase , inhibition of this enzyme leads to elevation of urinary coproporphyrin and accumulation of protoporphyrin in the erythrocytes which rise when blood Pb levels reach 150-300 μg per liter (Rogan, 1986 ; Phannee Pidetcha *et al.*, 1990).

Some of the mentioned enzymes and proteins in heme synthesis or some of its intermediates have now been used routinely as the biochemical indicators of Pb exposure , in accompanied by Pb concentration itself (Nakao, 1968; Rogan, 1986 ; Lolin and O'Gorman, 1986 ; Phannee Pidetcha *et al.*, 1990). However, the amount of sample is one of the limiting factor of this diagnosis. Moreover , it takes a certain period of time after the exposure , for significantly detectable change of these indicators (Phannee Pidetcha *et al.*, 1990).

Chisolm (1971) reported that Pb inhibits enzyme activity in some steps of heme synthesis (as shown in Figure 1). Furthermore, in 1986 Simons reported that it can also reduce iron transportation from plasma to erythrocytes. It is possible that the anemia caused by Pb may be due to the blocking of iron transportation by Pb.

1.4 Iron Absorption and Transportation

The metabolism of iron is chiefly regulated by the amount absorbed through the intestine which depends principally on the body needs (Crosby, 1965). Crosby, on the evidence obtained from experiments in rats, has suggested that absorption of iron is controlled both at its entry into the mucosal cell and its transport through the cell (Figure 2). Under normal physiological conditions , a small fraction of the iron in the lumen enters the mucosal cell and a portion of this is transported across the cell to the circulation and the rest is held in the cell as ferritin. In iron deficiency , the fraction of iron entering the epithelial cell is greater and almost all of this is transported to the circulation , practically none being retained as ferritin (Talwar, 1980).

When iron enters blood stream, it combines to transferrin , a glycoprotein of molecular weight about 90,000 dalton in plasma which exhibits a much greater affinity for iron than do the other plasma proteins, and be transported to bone marrow and to other compartment (Talwar, 1980). Most of this iron, available in the form of transferrin or ferritin, incorporated into the nucleated red cells of bone marrow and also to reticulocytes, find its way into the hemoglobin of newly born red cells which are soon found in the circulation (White *et al.*, 1968) ; smaller amounts go to the liver and spleen. This iron insertion

occurs in the last step of heme synthesis as shown in Figure 1. The homeostatic mechanisms involved in iron metabolism are summarized in Figure 3.

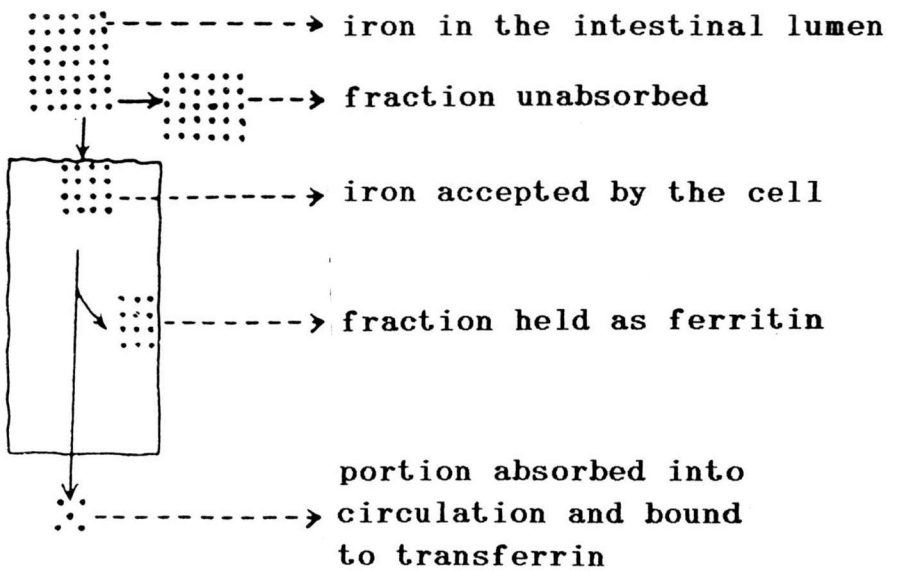

Figure 2 The absorption of iron into the cell and its transport through the mucosal cell (Talwar, 1980).

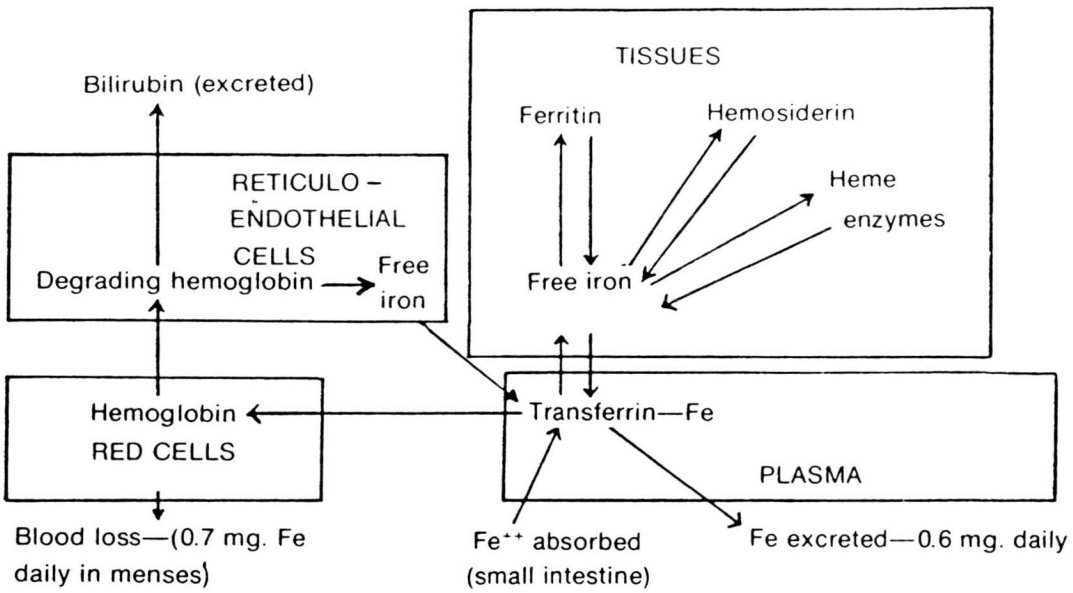

Figure 3 Iron transport and metabolism (Guyton, 1981).

1.5 Possible Route of Lead Transportation

As mentioned earlier , symptoms associated with various organs are common in Pb-toxic patients, but the picture explaining Pb transport in blood circulation is still unclear. This study is, therefore, aimed to make the mechanism clearer , wishing that the better understanding on Pb transport in blood circulation may lead to prevention and/or treatment of Pb poisoning.

Iron and other metals uptaken from food and environment are mostly transported throughout the body in

bloodstream (White *et al.*, 1968 and Talwar, 1980). Blood circulation is, therefore, the first biological system being contacted by these metals. Biochemically , the transport process is divided into 2 catagories : non - mediated transport occurs through simple diffusion , which is in contrast to the mediated transport through the action of specific carriers (Voet, 1990). When the transportation of essential metals is surveyed, it is evident that though some of them are transported freely in blood plasma, most of them are bound and cotransported with their specific carriers (Table 2 ; Frauste da Silva and Williams, 1993). However, literature survey points out that the study on transportation in blood circulation of Pb , foreign metal of the body , is unclear. After Clarkson and Kench (1958) reported that Pb is rapidly taken up by red blood cells, Simons (1986) reported that it can cross the membrane with the passive transport mechanism. The mobility and distribution of Pb within the body will , to some extent, be determined by the rate of transport across the red cell membrane (Simons, 1986).

It is worthy mention again that Pb can reduce heme synthesis which finally lead to anemia (Chisolm, 1971). The reduction of heme is caused either by the inhibition of enzymes in heme synthesis or by the impairment of iron transport. In the latter case , it is possible that Pb may interfere with iron transportation at the site of heme

synthesis via transferrin, the glycoprotein which has been clearly shown to be a specific iron-carrier in blood plasma and also serves as a key factor for the uptake of iron into the erythrocyte (Frauste da Silva and Williams, 1993). Gathering from these information, it is thus postulated that transferrin carries Pb in blood stream and into the erythrocytes.

Table 2 Carrier in blood plasma.

Metal ion	Carrier
Iron (and manganese)	Transferrin
Copper	ceruloplasmin
Zinc	An albumin
Cobolt (as vitamin B_{12})	Carrier protein
Calcium	Phosphoproteins (when needed)
Magnesium, sodium, potaasium	None

Aims of This Thesis

To prove the statement mentioned above , this research will be performed on human blood with the emphasis on the following aspects:

1. To study the uptake of Pb from human serum into red blood cells.

2. To point out , with *in vitro* experiments and in physiological condition, the target molecule in serum which takes important part in the transportation of Pb.

3. To check the effect of some chelators on the release of Pb from the target molecule.