CHAPTER I

INTRODUCTION

Background of the Problem

Lead was among the first metals known to the early Egyptians. It played an important role in the civilization process of some industries.[14] The chief use of lead is in the manufacture of storage batteries which accounts for approximately one-third of the total industrial lead consumption.[15]

As is true for many other substances which man has adopted to his use, lead may be harmful as well as beneficial. The toxic action of lead was first recognized by Hippocrates, who described a severe attack of colic in a man who extracted metals. By the first century A.D., lead poisoning was a well-recognized clinical entity. Early descriptions referred to individuals obviously exposed to large amounts of lead. It was not until the seventeenth century that the more subtle forms of lead poisoning was appreciated.[38]

At the present time the manifestations of lead poisoning are well documented but the laboratory tests used for its diagnosis have not yet been clearly defined. The laboratory tests which are used in the control of lead hazard may be classified into two categories, those which measure lead absorption and those which measure the effect of lead on tissue enzyme. The former includes the determination of lead in blood and urine and the latter includes hemoglobin level, urinary coproporphyrin (CP), delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) levels.[33,79,88]

The determination of lead in blood and urine is a difficult procedure requiring expensive instruments which are available only in certain laboratories. However, blood and urine lead concentrations may still be the best parameters to indicate the magnitude of lead absorption.[53,83,90,91] Several studies have been made correlating the different tests of lead absorption with the effect of lead on heme synthesis,[6,19,20,22,26,33,70] but the results from these studies have not always been in agreement.[88]

The main purpose of this study is to determine the simplest and most feasible method for the diagnosis of lead hazard by employing single voided urine which can be easily obtained. The attempt will be made to evaluate the merits of single urinary parameter by comparing with urinary lead concentration.

Literature Reviews

1. Metabolism

1.1 Absorption

About 10% of ingested lead, whether from food or water is absorbed from the intestine in adult man,[44,54,62,71] whereas 53% is absorbed in children.[52] The work of Kostial *et al.*[47] has shown that the lead absorption from the intestine in newborn rats is fifty times that of the adult rats.

There are significant differences in the absorption of different lead compounds. Compounds which are soluble or able to be converted to soluble forms in the gut are most readily absorbed.[4]

Nutritional factors have a marked effect on the absorption of lead from gastrointestinal tract. Milk has been found to increase lead absorption from the gastrointestinal tract in rats.[45] Calcium and phosphorus added to rat diets decrease the accumulation of lead in femurs and kidneys.[72,74] Conversely, low dietary iron has been shown to enhance lead uptake from the gastrointestinal tract in rats.[73] Protein has also been found to affect lead absorption and toxicity.[2] Barltrop et al.[3] found that lead absorption was increased by high fat, low mineral, low protein and high protein diets, but was decreased by high mineral diet. Low fat, low fibre, high fibre, low vitamin and high vitamin diets have no effects on lead absorption.

The intake of lead by respiration is very difficult to measure because it depends not only on atmospheric lead concentrations, but also on the volume of air inhaled by an individual. The daily respiratory intake of lead may vary from 1-60 µg.[77] The data of Nozaki[61] suggested that for particle size equivalent to those found in ambient air, the deposition of lead in the respiratory tract was approximately 37% of that was inhaled. It has been assumed that all the lead retained in the respiratory tract is eventually absorbed but there are no data for the rate of absorption.

Lead absorption through the human skin was also reported by Hine et al.[43]

1.2 Distribution

After absorption, lead is distributed as lead diphosphate to the soft tissue, especially brain, lungs, liver, spleen and marrow, from

these it is transferred to the bone where it is stored as the relatively insoluble lead triphosphate.[13] Lead concentration in bone greatly exceeds the concentrations in soft tissues. Approximately 80-90% of the human body burden of lead is found in bone, of which over 70% is in dense bone. Bone lead concentrations increase with age in both sexes. Male adults contain over 30% more lead in their bones than females.[5]

Blood lead makes up approximately 2% of the total body lead pool.[65,79] Approximately 90% of blood lead is bound to erythrocytes and is not readily diffusible.[12,71] The plasma lead, approximately 0.2% of the total body lead pool, is made up of two fractions, the plasma protein bound fraction and the diffusible fraction.[71]

1.3 Excretion

The ingested lead is mostly eliminated in faeces and urine. The daily retention is normally less than 10% of the total lead intake, therefore more than 90% is effectively eliminated from the body.[81]

The long term studies suggest that the faecal and urinary excretions are approximately equal in reducing the lead burden. Biliary excretion is much greater than the other forms of gastrointestinal excretion. Milk, sweat and hair losses are secondary routes of excretion.[15]

Urinary lead excretion is by glomerular filtration and possibly tubular secretion of the diffusible plasma lead fraction. In subjects with normal or mildly increased body lead stored, lead is excreted almost entirely by glomerular filtration. The relative importance of filtration and secretion in subjects with heavy lead exposure is unknown, but it is likely that a great part of the filtered lead is reabsorbed and then

secreted by the tubular lining cells.[65,86]

2. Lead and heme synthesis

Much of the early work concerned the effect of lead on heme synthesis. As early as 1895 as cited by White,[89] Stokvis showed that, in lead-poisoned rabbits there was an increased excretion of porphyrins. Later, increased excretion of coproporphyrin and delta-aminolevulinic acid was found in man.[40] It is now known that lead inhibits enzyme delta-aminolevulinic acid synthetase and dehydratase, coproporphyrinogen oxidase and heme synthetase.[15,28,36] The mechanism of inhibition is thought to be due to blockade of sulfhydryl (-SH) groups of these enzyme molecules.[15,89]

These changes and their results, which serve as the basis for the clinical pathologic changes and are important in diagnosis of lead poisoning, are illustrated in Figure I. The various urinary parameters proved to be useful diagnostic tools in lead poisoning are listed in Table 1.

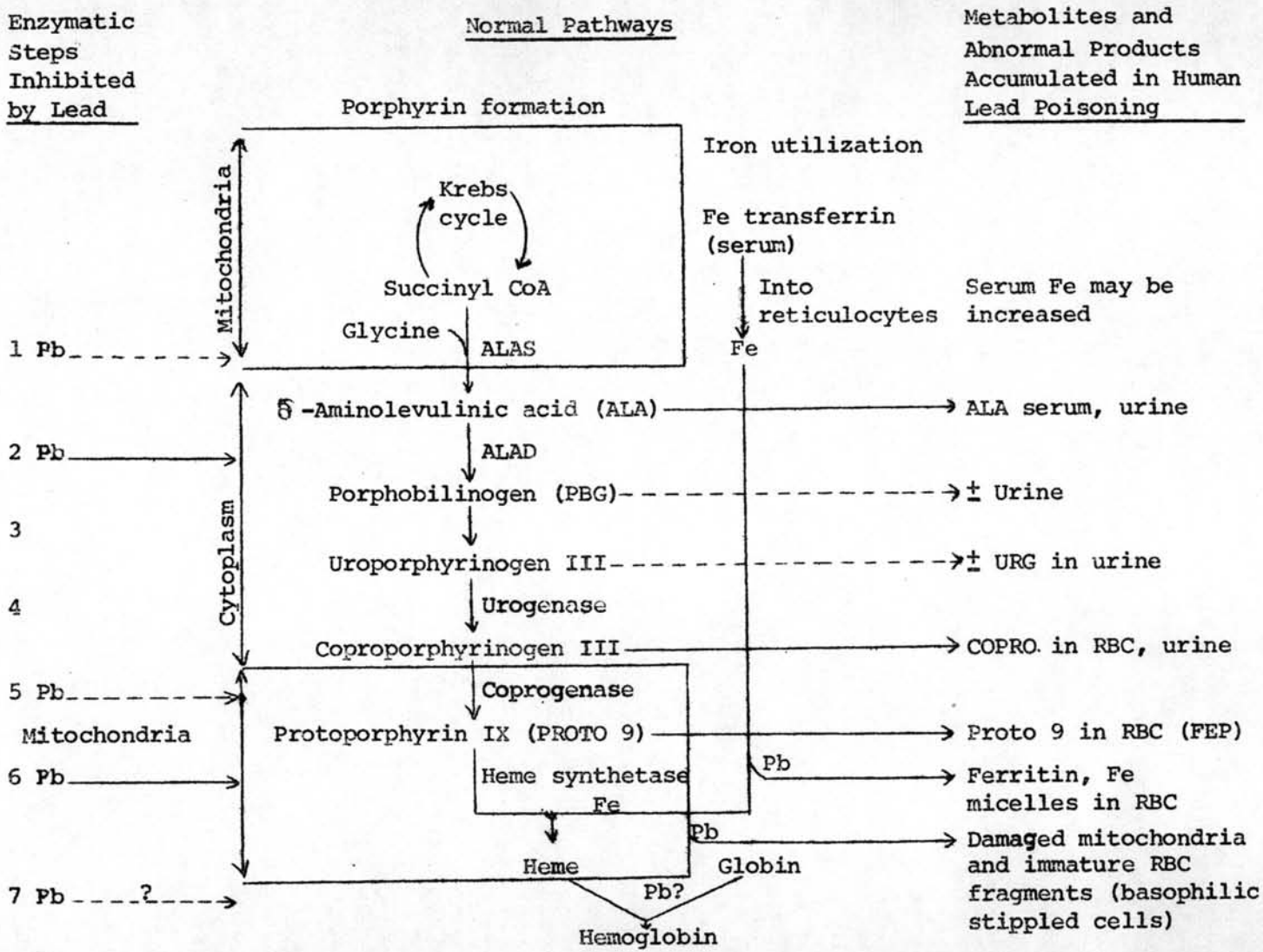

Fig. I. Interference of lead on heme synthesis at several enzymatic steps, on the utilization of iron, and on globin synthesis in erythrocytes.[15]

Table 1

Clinical Pathologic Diagnosis of Lead Poisoning*

Parameters	Normal	Acceptable	Excessive	Dangerous
Urinary lead (PbU)	<80 ug/l	80-150	150-250	>250
Urinary copropor-phyrin (CPU)	<150 ug/l	150-500	500-1500	>1500
Urinary delta-amino-levulinic acid (ALAU)	<6 mg/l	6-20	20-40	>40

*From Lane et al.(50)

3. Lead intoxication

The intoxication of lead may be divided into two stages:(88)

(a) Subclinical intoxication is an increase in lead absorption without signs and symptoms. The absorbed lead will show an interference with heme synthesis and will be detectable by the decrease of enzyme delta-aminolevulinic acid dehydratase activity and the excessive excretion of heme precursors in the urine.

(b) Clinical intoxication (lead poisoning) is an increase in lead absorption with an exhibition of signs and symptoms.

The symptomatology of lead poisoning may be divided into 3 forms as follows:[8]

(a) An alimentary form in which colicky pain as a result of bowel spasm is the primary feature.

(b) A neuromuscular form in which weakness of a muscle group precedes actual paralysis.

(c) An encephalopathic form which manifests itself in coma, convulsion, mania or delirium.

Symptoms common to all forms are anemia, insomnia, headache, dizziness and irritability.

Acute poisoning is very rare, because large quantities of lead are only slowly and not easily absorbed by the gastrointestinal tract. However, acute poisoning may occur, especially in children, following ingestion of a large amount of soluble lead compounds. The symptoms in acute poisoning are sweet metallic taste, salivation, vomiting, intestinal colic, retention of stool and urine, beginning damage to the blood, and collapse with fall in blood pressure and subnormal temperature.[15,59]

Another type of acute poisoning has followed industrial exposure to tetraethyl lead vapor which rapidly penetrates through the intact skin. The chief derangement here occurs within the central nervous system and manifests itself as insomnia, headache, restlessness, irritability, ataxia, delusions, mania and sometimes convulsions.[35]

Chronic poisoning is usually caused by exposure to small amount of lead over a long period of time. The symptoms are categorized as follows:[15]

Central nervous system: encephalopathy, fatigue, headache, tremor, hallucinations, intellectual deterioration, cortical atrophy, hydrocephalus, blindness, convulsions.

Gastrointestinal system: colic, loss of appetite, nausea, vomiting, constipation.

Hematologic system: hypochromic normocytic anemia, basophilic stippling erythroblasts, binucleated erythroblasts, increased serum iron.

Renal system: hyperuricemia, nephritis, glycosuria, hyperaminoaciduria.

Others: gum lead line (black or purplish line of gum margin), skin pallor (ashen gray), loss of weight, weakness of extensor muscles (wrist or foot drop).

Colic is one of the more common symptoms of chronic lead poisoning. This sign along with constipation and slight but detectable weakness of the upper extremities, wrists and fingers, is usually the earliest symptom. The lead encephalopathy seen today is rarely from industrial exposure. It occurs in children, particularly from the ingestion of lead-containing paints.

In Thailand, there were several case reports about lead poisoning. Trishnananda et al.[84,85] reported three cases of chronic lead poisoning from printing industry and two cases from melting and refining factory. The chief symptoms were nausea, vomiting and colicky pain, which in some cases were associated with headache and muscular weakness of extremities.

4. History of lead intoxication

Lead intoxication, one of occupational diseases is still reported by several authors. It is also a current disease found in many countries around the world. As we know, lead may get into the body by these routes, namely: inhalation of lead fume, ingestion of lead compounds and skin absorption of organic lead.

In Chicago, Mellins and Jenkins[58] reported 21 cases of lead poisoning in children during 1953. The source of lead in these cases was pica. Lead contamination of wine and grape syrup from the use of lead containers in Rome was reported by Gilfillan.[34] The contamination of liquids stored in glazed earthenware containers was also described by several investigators.[12,18,46,71] The contamination of a large area in Yugoslavia by lead from a mine and smelter was found by Djuric' et al.[27] Two cases of chronic lead poisoning due to the ingestion of contaminated opium were reported by Chia et al.[17] Lead poisoning in lead workers from various industries was also explained by Gibson et al.[33]

Bacon et al.[1] described three cases of lead poisoning due to long-term ingestion of soft water which had eroded lead from the inside of lead pipes bringing water to their houses. A small epidemic of lead poisoning was detected in Thai families using automobile battery cases as fuel in making coconut palm sugar at homes.[48] Caprio et al.[16] found that excessive lead absorption was increased in children who lived near the major highways and urban or heavy traffic roadways. Lead poisoning in the employees of the secondary lead smelter during 1975-1976 was reported by Maddock et al.[54] The outbreak was related to inadequate ventilation

of rotary furnaces used for lead processing.

Beattie et al.[7] also described four cases of lead poisoning in subjects simultaneously exposed to tetraethyl lead during the process of scaling a tank which contained leaded petrol. An accidental lead poisoning in a man exposed to a high level of tetramethyl lead which is blended with petrol as an antiknock agent was found by Gething.[32]

5. Correlation between some parameters of lead absorption and lead intoxication

In 1965, Cramér and Selander[19,20] studied 15 cases of lead poisoning with a wide range of biochemical test results and found correlation coefficient of 0.68 between urinary lead and urinary ALA, of 0.72 between urinary lead and urinacy CP and of 0.65 between urinary ALA and urinary CP. They also treated the subjects with penicillamine and observed the correlation between blood lead, urinary lead, lead excreted during therapy and urinary ALA. They found very strong correlation between blood lead and lead excreted during therapy ($r = 0.89$, $p < 0.001$), between initial values of lead in blood and ALA in urine ($r = 0.90$, $p < 0.001$), between lead in blood and ALA in urine during the course of therapy ($r = 0.89$, $p < 0.001$), but weak correlation between therapeutically excreted lead and initial values for lead and CP in urine. So it was concluded that the determinations of lead in blood and ALA in urine are equivalent as expression of lead poisoning, provided that the lead level in blood was not temporarily raised because of an acute exposure.

In 1967, de Bruin et al.[26] studied the correlations between the values of various parameters in individual workers and found high corre-

lation coefficients between ALA and CP excretion ($r = 0.85$), between CP and lead excretion ($r = 0.77$) and between ALA and lead excretion ($r = 0.79$)

In 1968, Gibson et al.[33] studied in 100 lead workers and found positive correlation between urinary CP and ALA ($r = 0.79$), no correlation between urinary CP or ALA and blood lead ($r = 0.16$) and no correlation between urinary CP or ALA and urinary lead ($r = 0.17$). Two years later, 117 workers of a storage battery factory were examined for lead in blood, lead and ALA in urine by Cramer and Selander.[22] The correlation found between blood lead and urinary lead was 0.66, between urinary lead and urinary ALA was 0.75.

In 1971, Waldron et al.[88] reported the correlations between various parameters of lead absorption and lead intoxication, including blood and urine lead concentrations, urinary CP, ALA and PBG concentrations, and hemoglobin concentration. In all, 15 correlation coefficients were calculated, of which only six showed statistically significant correlations ($p < 0.05$). These six were blood lead and urinary lead ($r = 0.38$, $p < 0.001$), urinary lead and CP ($r = 0.42$, $p < 0.001$), urinary lead and ALA ($r = 0.43$, $p < 0.001$), urinary CP and ALA ($r = 0.75$, $p < 0.001$), urinary ALA and PBG ($r = 0.49$, $p < 0.001$) and urinary lead and PBG ($r = 0.19$, $p < 0.05$)

Haeger-Aronsen[41] also reported that the correlation of ALA and CP in the urine was good. Both parameters were good indicators for the degree of lead poisoning. The former was more specific and more sensitive and was therefore considered the most suitable test for the biochemical monitoring of lead workers.

The recent work of Tola et al.[83] showed that urinary lead reflected blood lead fairly well, and could thus be regarded as a useful test of lead absorption. The correlations between urinary lead and other parameters were as follows; urinary lead and blood lead ($r = 0.58$), urinary lead and CP ($r = 0.48$), urinary lead and ALA ($r = 0.39$), urinary ALA and CP ($r = 0.48$)

6. Methods available for determination of lead in urine

Various analytical technics applying polarography, colorimetry or spectrography have been employed in the determination of lead in blood and urine.[11]

Polarography, though it is fairly suitable in industrial screening programs, lacks the sensitivity necessary to make it a useful tool in a clinical laboratory.

Colorimetric method is based on the reaction of lead and diphenylthiocarbazone (dithizone) to form a red complex in chloroform solution, which can be measured spectrophotometrically at 520 nm.[79] This method requires large amount of sample and is time consuming.

In 1961, Willis[93,94] used an atomic absorption spectrophotometric method for determination of lead in urine. Lead was chelated with ammonium pyrrolicidine dithiocarbonate (APDC) and extracted with methyl-n-amylketone, followed by vaporization of this solution in the flame of an atomic absorption spectrophotometer. The absorption of the lead line at 2833 Å was calibrated by extracting and measuring a similar quantity of urine to which a known amount of lead was added.

In 1968, Selander and Cramer[69] described a method for the determination of lead in urine by means of atomic absorption spectrophotometer by using a combination of wet ashing and extraction with APDC into methyl-isobutyl ketone (MIBK). The sensitivity of this method was about 0.02 µg/ml for 1% absorption and the detection limit was about 0.02 µg/ml with an instrumental setting convenient for routine analyses of urine samples. The results agreed well with those obtained by a colorimetric dithizone method (r = 0.989).

In the same year, Roosels and Vanderkeel[66] proposed a method which avoided digestion, for the determination of lead in the urine of patients on versenate therapy. The method was based on the property of calcium ions to replace lead in EDTA complexes. Urine was buffered with ammonium citrate, calcium chloride was added, and the lead was extracted with a chloroform solution of dithizone. The limitations of this method were the poor absorbance values obtained when chloroform was aspirated, and the necessity to use the 2833 Å line, which had less than half the sensitivity of the 2171 Å line.

In 1969, Farrelly and Pybus[30] also described a method for determination of lead in urine by modifying the method of Willis.[93,94] Fifty milliliters of urine were made acid with 10 milliliters concentrated hydrochloric acid and neutralized with 1 N NaOH using bromophenol blue as an indicator. Two milliliters of glacial acetic acid were added to buffer the urine to pH 2.6-3.0. Two tenths milliliter of APDC was added to chelate lead and the complex was extracted by 2.5 ml of MIBK. The MIBK solution was aspirated into the flame of an atomic absorption spec-

trophotometer. The absorbance was compared with that of the standard solution processed in the same manner. The recovery of lead added to urine specimens of patients on chelating agents was 100.2%.

In 1971, Matousek et al.[56] described a flameless atomic absorption spectroscopic technic. Samples were injected into a cavity drilled transversely through a carbon rod, and subsequently dried, ashed and atomized by the application of electric current. The resulting brief absorption signal was recorded and compared with that of standard.

In the same year, Zinterhofer et al.[95] also described the atomic absorption method for the determination of lead in blood and urine without prior acid digestion of specimens from patients receiving EDTA therapy. Lead was completely extracted as the APDC chelate in the presence of EDTA by the addition of an excess of calcium, which displaced lead from its EDTA complex. The entire procedure was performed in a single tube and provided sufficient concentration of lead to yield adequate absorbance values without scale expansion.

In 1973, Kubasik et al.[49] described a simplified determination of urinary lead by using carbon rod atomization and atomic absorption spectrophotometer. Lead was determined in a single 3 ml urine sample by chelated with sodium diethyldithiocarbamate. The complex was extracted, at pH 3-7, into MIBK solution. A carbon rod atomizer, in conjunction with an atomic absorption spectrophotometer, was used to analyse the extracts. The recovery of this method ranged from 96-106%. This method was used in this study because of its high sensitivity, accuracy and precision.

7. Methods available for determination of delta-aminolevulinic acid

ALA was first determined in the urine by Mauzerall and Granick[57] in 1956. The method required two ion exchange columns, Dowex 2 and Dowex 50, to separate ALA from PBG and other interfering substances which gave color with Ehrlich's reagent. The ALA eluate was reacted with ethyl acetone at pH 4.6 in a boiling water bath. The pyrrole formed was allowed to react with modified Ehrlich's reagent and measured colorimetrically at 553 nm. The recovery of this method was 90±2%.

In 1967, Davis et al.[24,25] modified the method for isolation of ALA from the urine by using disposable ion-exchange chromatographic columns in order to adapt the analysis of urinary ALA in a fashion which would be suitable for large scale screening purposes. The columns used were AG-1-X8 and AG-50-X4 which could retain PBG and ALA respectively.

In the same year, William and Few[92] also simplified the method of Mauzerall and Granick by using only one column, the Dowex 50. The correlation coefficient of the original and the simplified method was 0.99. The latter was recommended for screening lead poisoning but may give lower specificity and falsely high values in pathological conditions.

In 1969, Sun et al.[78] also modified the method for using a single ion-exchange column instead of two ion-exchange columns. The method consisted of removing the ALA from a single cation-exchange resin and treating the ALA with acetylacetone to form a pyrrole. This product was then complexed with DMAB (Ehrlich's reagent) to form a red compound. A correction factor was used for PBG and other interfering substances by treating an aliquot of the eluate directly with DMAB. The method was

accurate and save.

In the same year, Wada et al.[87] described a simple method for the quantitative analysis of urinary ALA by removing the interfering substances by n-butanol extraction. After pyrrole formation with ethyl acetoacetate, Ehrlich's reagent was added to produce the chromophore, which was then extracted with chloroform and measured spectrophotometrically or by comparison of the depth of colour with standard colour solutions. The recoveries were about 91% and the results agreed well with those obtained by using ion-exchange column chromatography (r = 0.985).

Urinary ALA was also studied using monodimensional thin-layer electrophoresis on cellulose layer.[67] The separated ALA was detected with a cupric nitrate ninhydrin reagent. The quantity of ALA was estimated by visual or densitometric comparison with the standards.

The recent method of Tomokumi and Ogata[82] allowed urinary ALA to condense with ethyl acetoacetate to form 2-methyl-3-carbethoxy-4-(3-propionic acid) pyrrole which was extracted from an aqueous solution with ethyl acetate. The colour resulted from the reaction of pyrrole extract and Ehrlich's reagent would be quantitated colorimetrically. This method is suitable for the routine determination of urinary ALA in large numbers of specimens. It is a rapid, inexpensive and accurate procedure. Therefore, it was used in this study.

8. Methods available for determination of urinary coproporphyrin

The method for determination of urinary CP was introduced by Schwartz et al.[68] Ethyl acetate was used for extraction and the extract

was then washed twice with sodium acetate to remove interference and with aqueous iodine to convert nonfluorescent coproporphyrin precursor to fluorescent form. The porphyrin was then extracted with 1.5 N hydrochloric acid and analysed by fluorometric method. This method was reproducible with an error of 3-4%.

Zondag and Kamper[96] described a spectrophotometric method for the quantitative determination of coproporphyrin. Urine was adjusted to pH 5.0-5.5 and extracted twice with ether-glacial acetic acid mixture (100:5). The ether layer was washed first with 1% sodium acetate and then with water. The coproporphyrin was extracted from the ether layer with 0.1 N hydrochloric acid. The acid extract was measured colorimetrically.

Benson and Chisolm[10] reported a qualitative technic for the determination of urinary CP. Single voided urine was acidified to pH 4.0 with glacial acetic acid and extracted with equal volume of ether. The coproporphyrin in ether layer was extracted with 1.5 N hydrochloric acid, then was examined for fluorescence under ultraviolet lamp. The results were reported according to the intensity of fluorescence as 1+, 2+, 3+, and 4+ which corresponded to 1, 2, 3, and over 5 ug/ml respectively. The technic was sensitive enough for clinical use.

In 1966, Fernandez[31] incorporated an alumina column chromatography into the assay method of urinary CP in order to separate CP and uroporphyrin from ALA and PBG. The separated CP was then extracted with ether, acidified and determined colorimetrically at 380, 430 and 401 nm. The concentration was calculated and corrected for background absorption using Rimington Formula.[63] This method was compared to the previous extraction method

and the results were found to be similar. The normal range was reported to be 0-161 ug/day.

Martinez and Mills[55] also described a rapid method for determination of porphyrin in urine by using Dowex 1x8 anion-exchange resin and spectrofluorometer. Normal mean value for porphyrin was found to be 0.063 umole/l ranging from 0.028-0.176 umole/l. "Porphyrin" by this method meant the combination of CP and uroporphyrin.

Sobel et al.[75] modified the method by using Dowex 1x2 to separate CP from uroporphyrin and determined their concentrations spectrofluorometrically. The ranged values of the CP and uroporphyrin were 35-235 and 15-60 ug/24 hours urine respectively.

In 1974, Soulsby and Smith[76] modified Rimington's method[64] by extracting and oxidizing CP from ether layer with acid-iodine solution and determined the concentration spectrophotometrically at 380, 401 and 430 nm. After applying correction factor for the modified method, the correlation coefficient with the original method was 0.986. This method was selected to be used in this investigation due to its simplified procedure.

Purpose of the Study

The purpose of this study is to determine the simplest and most feasible method for the screening of lead workers by employing single voided urine.

The study will comprise of:

(a) The comparison of urinary lead, urinary coproporphyrin and urinary delta-aminolevulinic acid between occupational lead workers and control group, expressed both in unit per volume of urine and unit per gram creatinine.

(b) The correlation of urinary lead concentration and the other two parameters.