TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

IN VITRO EFFECTS OF LEAD OXIDE ON THE HUMAN ERYTHROCYTE

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Biology Extended Essay

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Candidate number : dhp930(001129-089)

Word Count : 3977

Abstract

Lead has no biological function; however, low, and particularly, high levels of exposure have a number of negative consequess for human healt. Despite the number of reports about lead tocicity, very little information has been obtained regarding its effects on erythrocytes. For this reason, the morphological effects of lead on human erythrociyte were investigated.

Four lead and four no lead crystal glasses were bougth and divided two groups each other. After completing the decontamination process, 200 ml of blood, or water were poured into one of the four lead and no lead crystal glasses. We measured concentrations of lead that leached into water and blood that were stored in lead and no lead crystal glasses for 1-, 2-, ….and 10- day periods at room temperature. Lead concentrations in the liquid matrix were measured using atomic absorption spectroscopy. Significant amounts of lead leached into the liquid within one day; 724 µg/L in blood, 820 µg/L in water. Lead continued to leach into both blood and water with the passage of time; 1832 µg/L in blood, 1653 µg/L in water (10^{th} day results). Lead release was less in water than blood. Light microscobic examination of peripheral blood smears from no lead glasses relevead no morphologic changes over the erythrocytes but peripheral blood smears from lead crystal glasses showed basophilic stippling. We found four basophilic stippling at 22 lead crystal glass peripheral blood smears (18%). Significant lead contamination of water was detected when it was left in lead crystal glass. If a liter of contaminated water was drunk daily, the daily intake of lead could have been as high as 1653 µg. Such a high degree of contamination could cause chronic lead poisoning and anemia. It is recommended that we should avoid all lead based glassware.

Word Count: 293

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Introduction

Crystal glasses have very good shape and designs. I wonder the difference between crystal glasses and the normal glasses. When I searched the distinction between them; I learned that crystal glasses contain some percentage of lead. The reason for this was to give shape. Lead is the most suitable heavy metal for giving shape. Therefore manufacturers use lead to produce these beautiful crystal glasses. As we know that lead is an heavy metal and have the potential for intoxication when it is taken over some cut-off levels. With this point of view, I thought that whether drinking or storage of liquids in the crystal glasses would cause any lead intoxication by leaching into the liquids. As a result I decided to test this possibility.

Lead is an environmentally persistent metal that has been redistributed in the environment as a result of human activities over thousands of years (1). Lead is present in our environment in water, soil, dust and products manufactured with lead (2). It has been used in construction, for decoration, and even as a food additive. It also has been a known health risk for centuries (3). Hippocrates is thought to have written the first case report of lead poisoning in 600 BC. The Romans also were aware of the toxicity of lead, with Pliny, Paulus Aegineta, and Vesuvius all commenting on its effects (4). As a summary usage of lead and lead glasses have history of nearly 5000 years in the history of humankind (5, 6). Detailed information can be found in appendix 1.

1. Warniment C, Tsang K, Galazka SS. Lead poisoning in children. Am Fam Physician. 2010 Mar 15;81(6):751-7)
2. Patrick L. Lead toxicity, a review of the literature. Part 1: Exposure, evaluation, and treatment. Altern Med Rev. 2006 Mar;11(1):2-22. Review.)
3. Warniment C, Tsang K, Galazka SS. Lead poisoning in children. Am Fam Physician. 2010 Mar 15;81(6):751-7)
4. Aub JC, Fairhill LT, Minot AS, Reznikoff P, Hamilton A. Lead Poisoning. Medicine Monographs Volume 7. Baltimore, Md.: Williams & Wilkins; 1926.
5. http://en.wikipedia.org/wiki/Lead_glass
6. Benhima H, Chiban M, Sinan F, Seta P, Persin M. Removal of lead and cadmium ions from aqueous solution by adsorption onto micro-particles of dry plants. Colloids Surf B Biointerfaces. 2008 Jan 15;61(1):10-6. Epub 2007 Jun 30.

Lead glass contains typically 18–40 weight % lead oxide (7). Lead crystal ware may release lead into the food and beverages when it comes in contact with. As well, any container you drink from, including one made of lead crystal, that has an exterior decorative pattern around the rim, such as a coating or glaze, may also release lead from the coating or glaze. Lead can be harmful to your health (8).

Lead interferes with heme biosynthesis, and it affects formation and function of erythrocytes. Lead, furthermore, interferes with iron utilization for heme formation, and radio-iron studies showed that lead competes with iron for incorporation into erythrocytes. If lead was indeed toxic to the hematopoietic system, one would expect the risk of aplastic anemia might be associated with lead exposure (9).

According to the above information. I learned that lead is a toxic heavy metal that can give harms to many biological systems in our body. In our daily life, we usually use lead crystal glasses unconciously. Does the use of crystal lead glasses cause lead intoxication by the leaching of lead into beverages and liquids that we drink from those glasses? First of all, I tired to confirm the existency of lead in the fluids that are stored in lead glasses. This was the first step of my study. Does this amount of leached lead cause any damage on erythtrocytes and what are the types of these damages? The second part of the study is occurred after confirming the availability of lead in the fluids that I studied. As it is known lead may harm many biological systems. Blood is one of these systems. As a general information, lead exposure may cause anemia by distortion of erythrocytes. As a second part of my study, I tried to show toxic effects of lead on light microscobe (i.e. basophilic stippling, toxic granulation etc.).

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7. http://en.wikipedia.org/wiki/Lead_glass
8. Labbé RF. Lead poisoning mechanisms. Clin Chem. 1990; 36:1870
9. Emsley, John (2005). Elements of murder. Oxford University Press. ISBN 0192805991. http://books.google.com/?id=qBnfMimUoCYC&printsec=frontcover.)

Hypothesis

Lead was used for ceramic lead glazes. This material interdependence suggests a close working relationship between potters, glassmakers, and metalworkers (10). Items made of lead glass may leach lead into the food and beverages contained (11). Under conditions of repeated use of the decanter, the lead leaching steeply decreases with increasing use (12).

By the help of this information, it was hypothesized that the transition of lead will occur from crystallized products. Since lead have some important inhibitor role in the enzymatic production process of heme (Appendix 2); the leached lead in the liquids might have some detrimental effects on the erythrocytes.

Since the synthesis of heme is an important factor in my hypothesis, I will summarize the production of heme in short. Heme is the prosthetic group of hemoglobin, myoglobin, and the cytochromes. Heme synthesis occurs partly in the mitochondria and partly in the cytoplasm. The process begins in the mitochondria because one of the precursors is found only there (13).

Heme synthesis begins with condensation of glycine & succinyl-CoA, with decarboxylation, to form d-aminolevulinic acid (ALA). Then stops with the formation of the heme as shown the appendix 3**.** Lead inhibits the heme pathway in several steps. These are summarized in appendix 4 (14).

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10. Lin; Tan, DT; Ho, HH; Yu, CC. "Environmental lead exposure and urate excretion in the general population.". The American journal of medicine. 2002, 113 (7): 563–8. doi:10.1016/S0002-9343(02)01296-2. PMID 12459402.
11. "Lead Crystalware and Your Health". It's Your Health. Health Canada. http://www.hc-sc.gc.ca/hl-vs/iyh-vsv/prod/crystal-cristal-eng.php.
12. Barbee SJ, Constantine LA. Release of lead from crystal decanters under conditions of normal use. Food Chem Toxicol. 1994 Mar;32(3):285-8.
13. Layer G, Reichelt J, Jahn D, Heinz DW. Structure and function of enzymes in heme biosynthesis.Protein Sci. 2010 Jun;19(6):1137-61
14. Layer G, Reichelt J, Jahn D, Heinz DW. Structure and function of enzymes in heme biosynthesis.Protein Sci. 2010 Jun;19(6):1137-61.

In view of this information, it was hypothesized that liquids that are stored and dronk in crystal glassware contain lead. Amount of this lead increase in proportion to the storage time. Consumption of lead containing liquids may cause toxic effect on many organ systems (such as blood, brain etc.) following the absorption of lead from gastrointestinal tract. It is clear that lead passing to blood have detrimental effects on erythrocytes. In my opinion, if we perform peripheral blood smear to the lead containing blood, we can hope to see basophilic stipplings on erythrocytes that are the sign of lead intoxication.

The purpose of this investigation is to study the effects of low levels of Lead on erythrocytes in an in vitro study design.

Method Development and Planning

Planning

I will plan to make this study in a two step design. In the first step, I will research to detect any leaching from crystal glasses into drinking water and blood. In order to test this existency, I will pour water and blood into the lead glasses and then measure the levels of lead. In the second step; I will plan to investigate the potential detrimental effects of leach lead over erythrocytes such as basophilic stippling and toxic granulation by light microscobe.

I will perform this study in GATA Military Medical Faculty. I will identify people who can help me. In order to do this work, I will have a meeting with these persons.

I will meet with Assoc. Prof. Oral Nevruz, MD from Department of Hematology for hematological procedure. I will meet with Assoc. Prof. Ismail Avci, MD from Department of Blood Bank. I will want two bags blood from him. I will meet with Asist. Prof. Ayse Eken, MSci from Deparment of Pharmacology. I will want to help me with the usage of atomic absorption spectroscopy and measure Lead concentration. I will meet with Asist. Prof. Suat Doganci, MD from Department of Cardiovascular Surgery. I want to help me decontamination procedure and statistical analyses. I will meet with Sati Uludogan from Department of Radiology. I want to help me X-ray imaiging.

I will buy four lead crystal glasses and four no lead glasses representing one manufacturer. The metal composition of lead crystal glasses will 24% metallic lead. All compositions will be fabricated to hold approximately 200 ml of liquid.

For this study, drinking water and human blood will be used as the test liquid. Human blood samples will obtained from Department of Blood Bank, Gulhane Military Academy of Medicine, Ankara/Turkey. Water will be purchased from the market.

Before the beginning of the study period all of the eigth glasses will be undertaken in a decontamination process. After completing the decontamination process, I will begin the study protocol.

On day 0 of the study, 200 ml of blood, or water will be poured into one of the four lead crystals and four no lead crystal glasses. Five milliliters of liquids will be removed for lead measurement and periferal blood smear at before pouring and 1, 2, 3,…,10 days after the initial day of the experiment. For this process, I will buy 88 blood tubes and 44 peripheral blood smear materials.

All incubations will be at room temperature. After 10 days, we will collected lead leachate samples and measured lead levels with atomic absorption spectroscopy. Meassure of lead levels will be performed at the department of of Pharmaceutical Toxicology with the supervision of Asist. Prof. Ayse Eken, Msci.

Also, lead leaching into water and blood from crystal glasses will be tried to confirm by radiological imaging. Radiologic examination will be performed in Department

of Radiology, Gülhane Military Medical Academy, Ankara, Turkey, with the supervision of Sati Uludogan.

In the seceond step detrimental effects of leached lead over erythrocytes will be detected. This will be performed with light microscope by investigation of peripheral blood smears that will be prepared in 10 days period. Fourty-four peripheral blood smear will be prepared this process. Morphologic abnormalities of blood cells will be discovered by microscopic examination with the oil immersion lens of well-prepared films of peripheral blood stained with Wright's stain. Inspection of erytrocytes will have been done in GATA Hematology Deparment with the supervision of Assoc. Prof. Oral Nevruz, MD.

Data colection and Statistical analyses will be done by a SPSS (Chicago,IL, USA) statistical program. These procedure will be performed in Department of Cardiovascular Surgery, Gülhane Military Medical Academy, Ankara, Turkey. Asist. Prof. Suat Doganci, MD help me to have these findings.

Materials

- Four lead crystal glasses
- Four no lead crystal glasses
- 800 ml blood
- 800 ml water
- 44 blood sample tubes
- 44 water sample tubes
- 44 peripheral blood smear materials
- Atomic absorption spectroscopy
- Roentgenogram cassette
- Mobile X-ray machine
- Non sterile gloves
- Injectors
- Light microscope

Method

I planned to make this study in a a two step design. In the first step, I researched to detect any leaching from crystal glasses into drinking water and blood. In order to test this existency, I will pour water and blood into the lead glasses and then measure the levels of lead. In the second step; I planned to investigate the potential detrimental effects of leached lead over erythrocytes such as basophilic stippling and toxic granulation by light microscobe.

I thought to perform this study in GATA Military Medical Faculty. I have identified people who can help me. In order to do this work, I had a meeting with these persons. Asist. Prof. Suat Doganci, MD (Department of Cardiovascular Surgery), Assoc. Prof. Oral Nevruz, MD (Department of Hematology), Asist. Prof. Ayse Eken, MSci (Deparment of Pharmacology), Sati Uludogan (Department of Radiology), Assoc. Prof. Ismail Avci, MD (Department of Blood Bank) have valuable contributions in every step of my study.

First Step Study Method:

Figure 1. Crystal glasses

Four lead crystal glasses and four no lead glasses representing one manufacturer (Paşabahçe®, Turkey) were bought (Figure 1). The metal composition of lead crystal glasses was 24% metallic lead. All compositions were fabricated to hold approximately 200 ml of liquid, and their dimensions were as fallows; upper diameter 11 cm, lower diameter 8 cm and height 4 cm.

For this study, drinking water and human blood were used as the test liquid. Human blood samples were obtained from Department of Blood Bank, Gulhane Military Academy of Medicine, Ankara/Turkey (Assoc. Prof. Ismail Avci, MD). Water was purchased from the market (Erikli®, Turkey).

Before the beginning of the study period all of the eigth glasses were undertaken in a decontamination process. The aim of this process is to prevent any possible unwanted material that can be on study glasses. Asist. Prof. Suat Doganci, MD adviced this decontamination process and we together applied this process (Appendix 5). After completing the decontamination process, I begin the study protocol.

On day 0 of the study, 200 ml of blood, or water were poured into one of the four lead crystals and four no lead crystal glasses. Five milliliters of liquids were removed for lead measurement and periferal blood smear at before pouring and 1, 2, 3,…,10 days after the initial day of the experiment (Figure 2, 3, 4, 5).

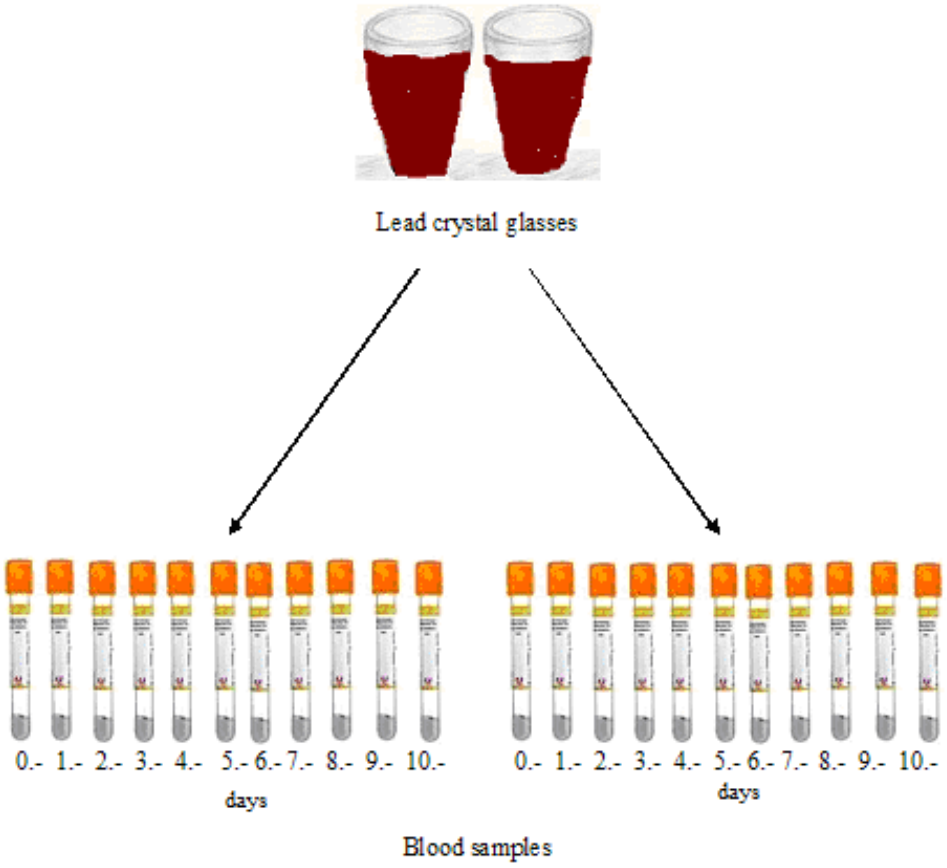


Figure 2. This figure explains the procedure of blood samplings from lead crystal glasses for a 10-day period. We collected 22 blood samples this process.

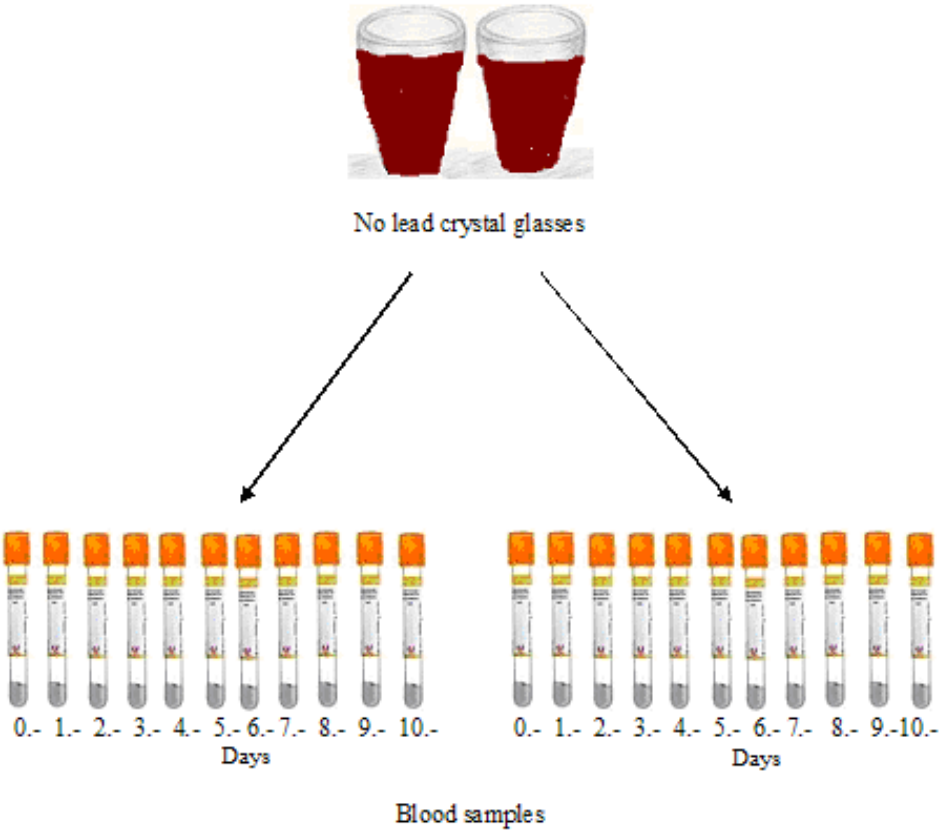


Figure 3. This figure explains the procedure of blood samplings from no lead crystal glasses for a 10-day period. We collected 22 blood samples this process.

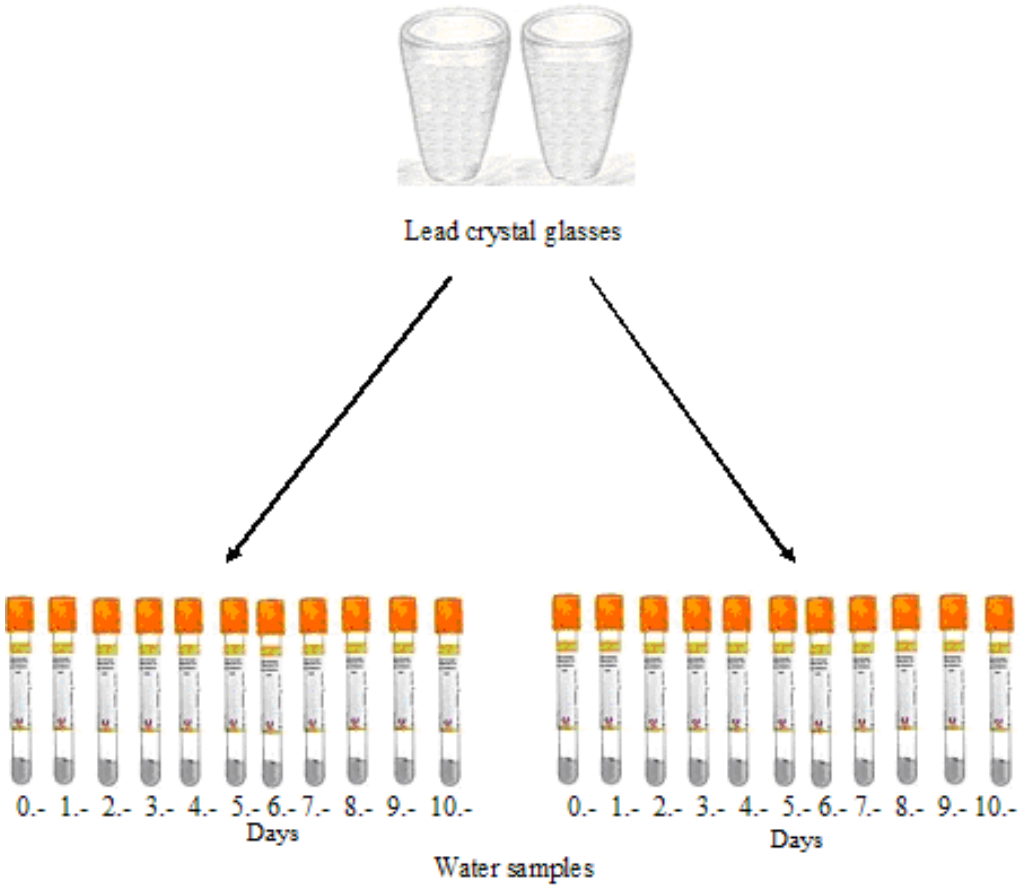


Figure 4. This figure explains the procedure of water samplings from lead crystal glasses for a 10-day period. We collected 22 water samples this process.

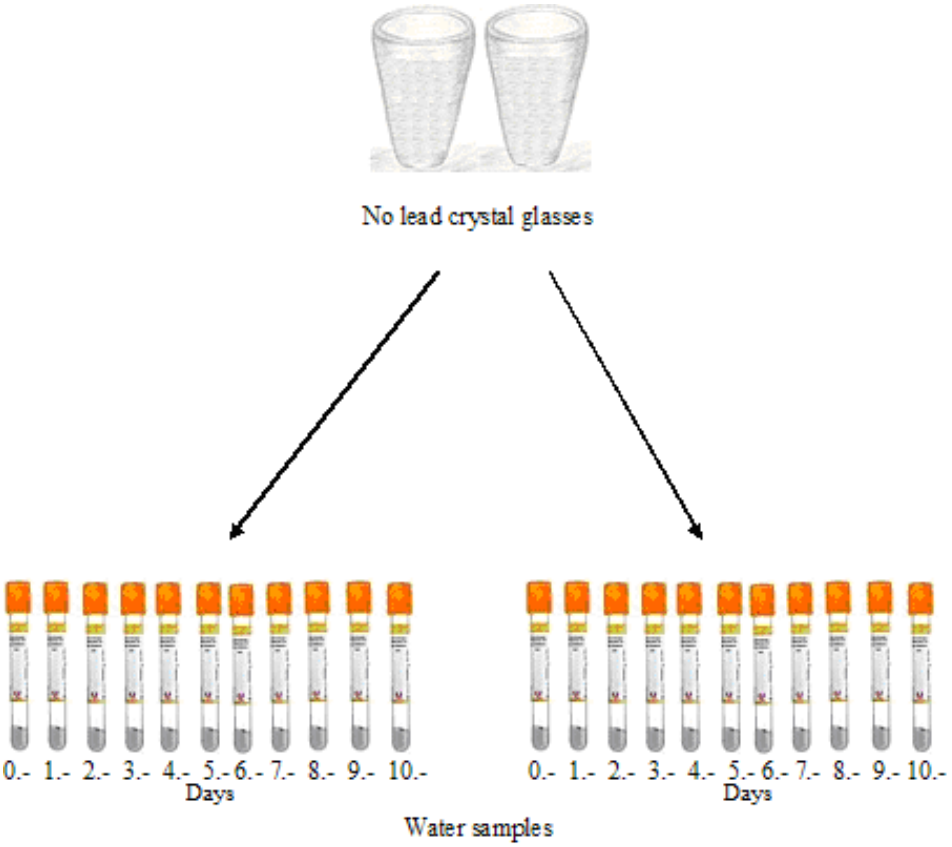


Figure 5. This figure explains the procedure of water samplings from no lead crystal glasses for a 10-day period. We collected 22 water samples this process.

All incubations were at room temperature. After 10 days, we collected lead leachate samples and measured lead levels with atomic absorption spectroscopy. Meassure of lead levels is performed at the department of of Pharmaceutical Toxicology, Gulhane Military Academy of Medicine. Asist. Prof. Ayse EKEN, MSci, help me with the protocol and we together measured the lead levels according to the following procedure. Detailed information can be found in appendix 6. Glass leaching standardization is described in appendix 7.

Also, lead leaching into water and blood from crystal glasses was tried to confirm by radiological imaging (Mobile X-ray machine, Philips, USA). This was done by having the X-ray image of four different objects (lead crystal glass, empty glove, glove containing blood (after 10 days of incubation), injector that contain water (after 10 days of incubation) over a roentgenogram cassette. Radiologic examination was performed in Department of Radiology, Gülhane Military Medical Academy, Ankara, Turkey. Sati ULUDOGAN help me to have the X-ray film.

Second Step Study Method:

In this step detrimental effects of leached lead over erythrocytes were detected. This was performed with light microscope by investigation of peripheral blood smears that were prepared in 10 days period (Figure 6, 7). Inspection of erytrocytes had been done in GATA Hematology Deparment with the supervision of Assoc. Prof. Oral Nevruz, MD. Detailed information can be found in appendix 8.

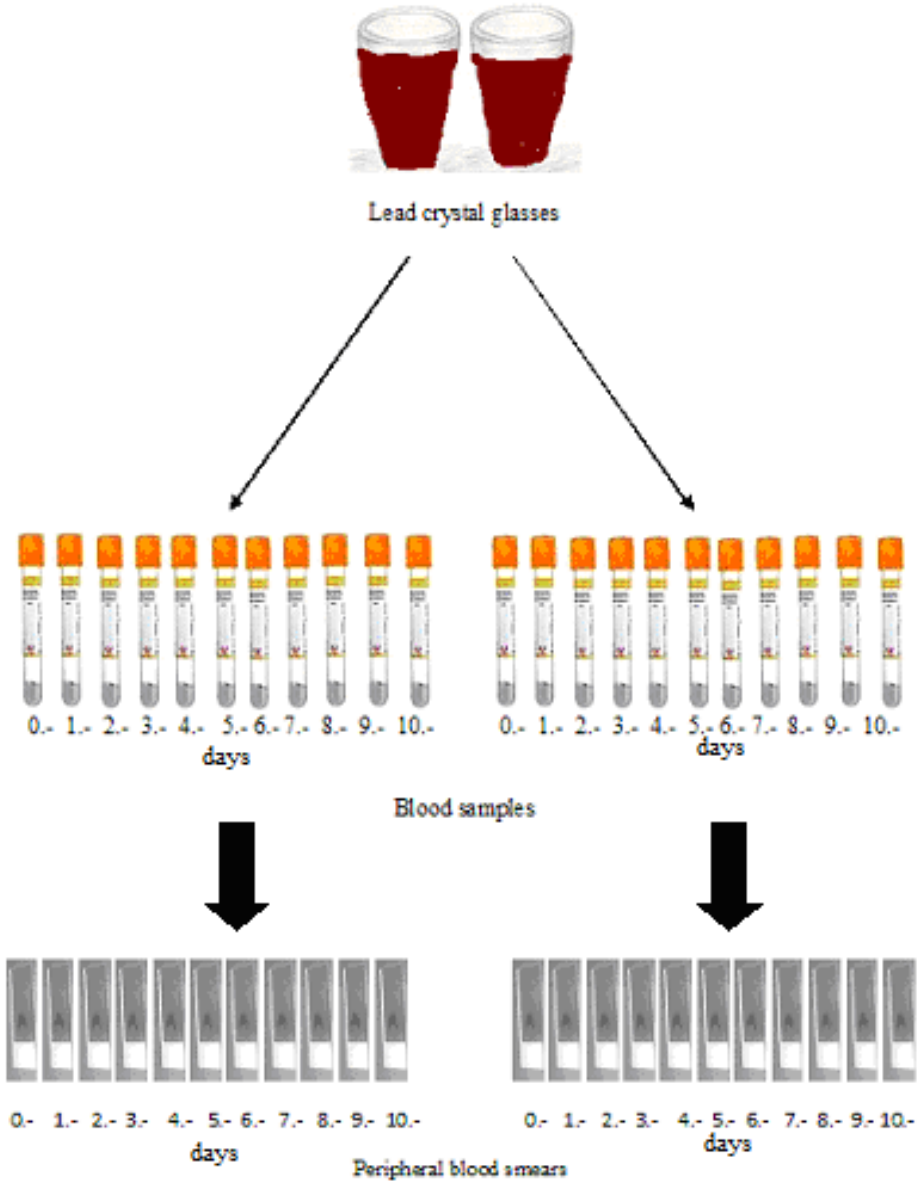


Figure 6. This figure explains the procedure of peripheral blood smears from lead crystal glasses for a 10-day period. We collected 22 peripheral blood smears samples this process.

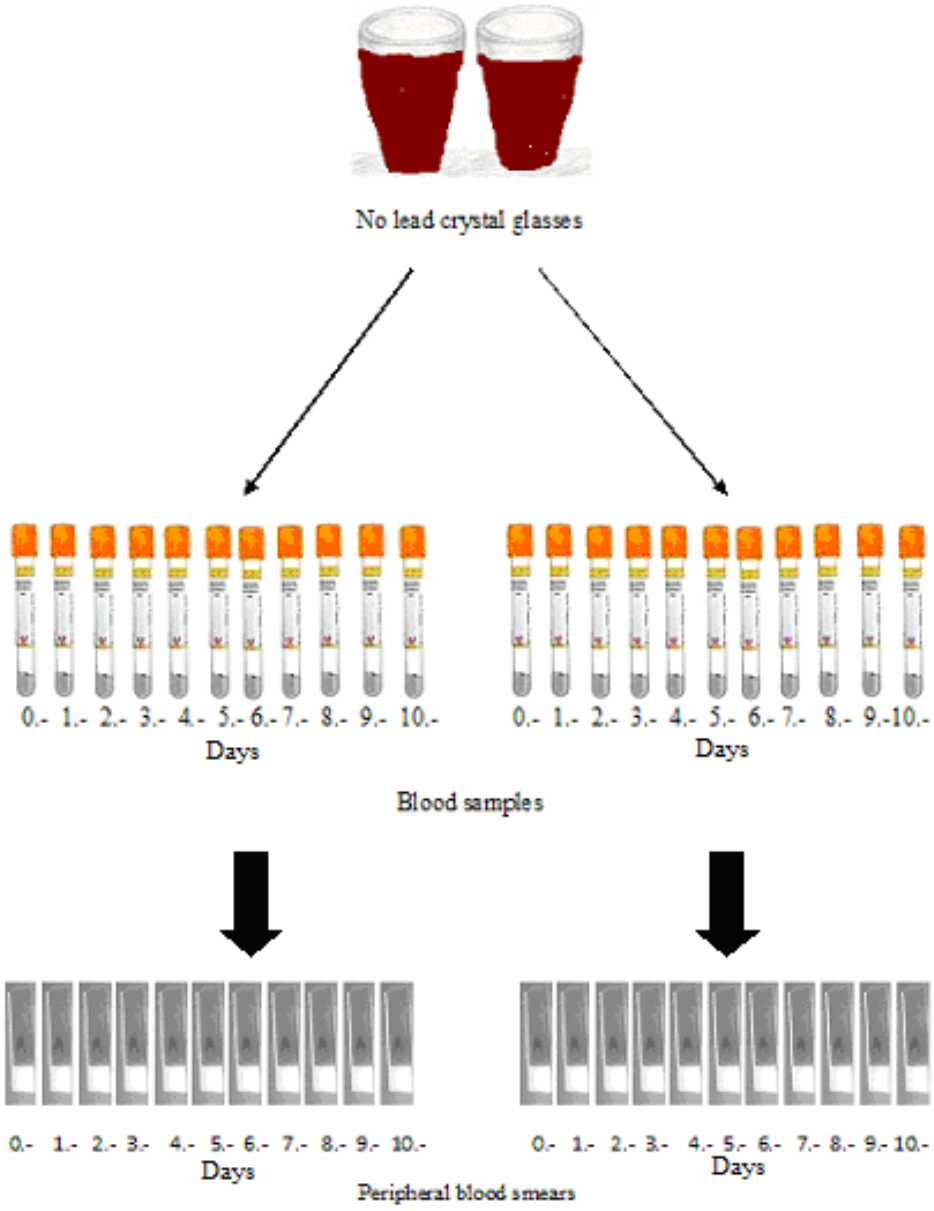
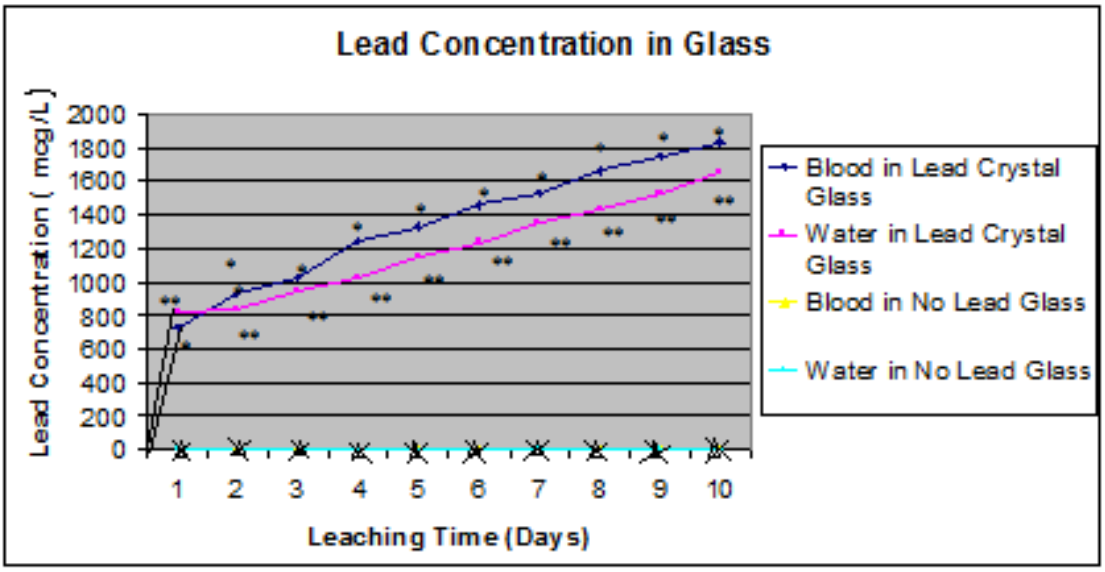


Figure 7. This figure explains the procedure of peripheral blood smears from no lead crystal glasses for a 10-day period. We collected 22 peripheral blood smears samples this process.

Data colection and Statistical analyses were done by a SPSS (Chicago,IL, USA) statistical program. Measured data are expressed as mean values ± standart deviation. Wilcoxon test was used for the comparison of measurements before and after the leaching period and morphologic abnormalities. A p value < .05 was considered significant. These procedure were performed in Department of Cardiovascular Surgery, Gülhane Military Medical Academy, Ankara, Turkey. Asist. Prof. Suat Doganci, MD help me to have these findings.

RESULTS

Four lead crystal glasses showed concentrations exceeding 1000 µg/L after 10 days, while four others (no lead glasses) showed concentrations of approximately 3 µg/L or less. In all samples, a rapid increase during the first 24 hours was followed by a prominent increase in lead concentration. Graphic 1 illustrates the change in leachate lead concentration over time for glasses.



*p<0.05, **p<0.05

Graphic 1. Lead concentration in glass

There was statistically significant difference between lead crystal glass and no lead crystal glass with leaching lead into liquid. There was no statistically difference between blood in lead crystal glass group and water in lead crystal glass with leaching lead. Table 1 lists all 10-day results, expressed as lead concentrations, as well as the total amounts of lead leached into each leaching agent, uncorrected for agent contribution.

Table 1. Summary of 10-day lead and no lead crystal glass leaching results.

Leaching agent	**Glass**	**[a]Leachate concentration (µg/L)**	**Total lead (µg/Leachate)**
Blood	Lead crystal glasses	1832±7*	1272
Water	Lead crystal glasses	1653±8**	1114
Blood	No-Lead crystal glasses	3±0.3	1
Water	No-Lead crystal glasses	0	0

*p<0.05, **p<0.05

In the study of periferal blood smear, basophilic stipplings (Figure 8) were seen. (In order to detect basophilic stipplings more efficiently, RBCs were packed with micro haematocrit method and after Wright-Gimsa staining, these cells were seen more clearly). Identification of basophilic stippling in peripheral blood smear and blood lead level of 1832 µg/dl were recorded with AAS at the 10^{th} day.

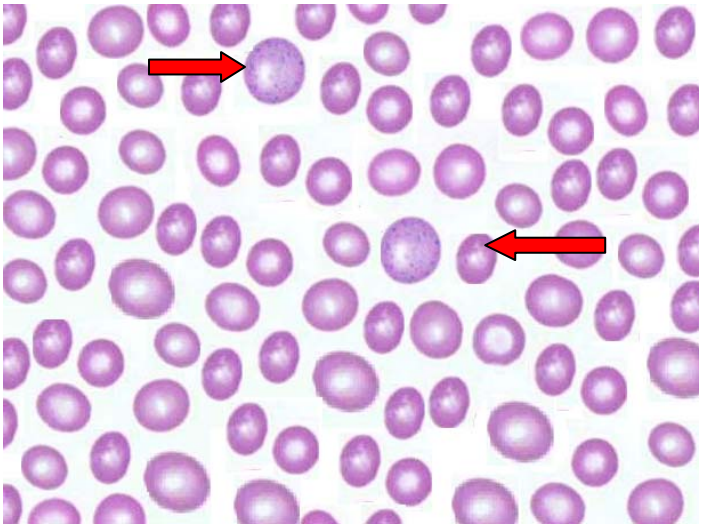
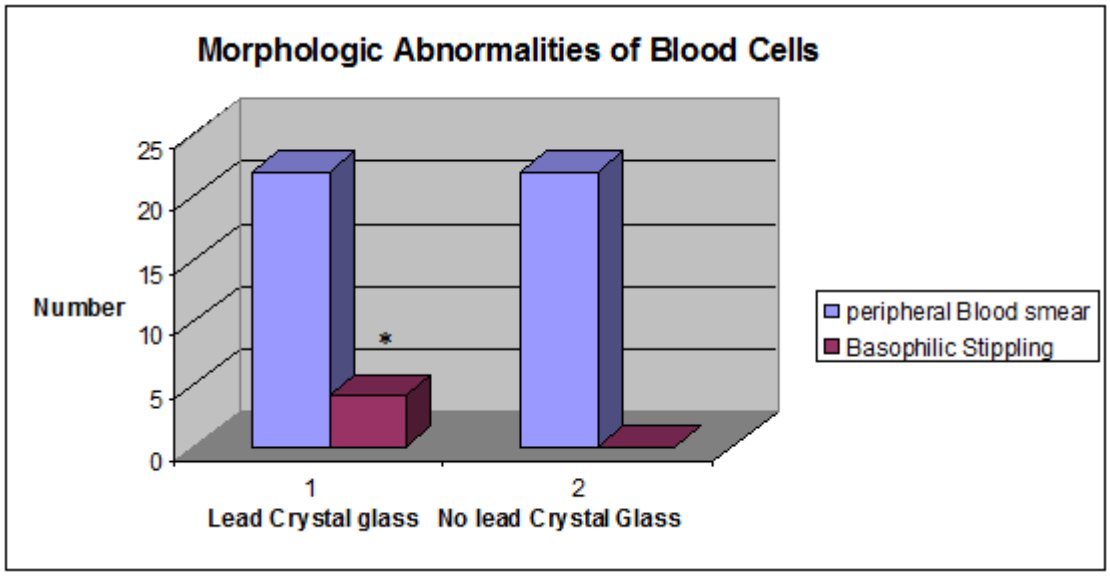


Figure 8. Peripheral blood smear from lead glasses showing basophilic stippling in the erythrocytes

Light microscobic examination of peripheral smears from no lead glasses revelaed no morphologic changes over the erythrocytes. There was statistically significant difference between lead crystal glass peripheral blood smears and no lead crystal glass peripheral blood smears with basophilic stippling. There was no observed basophilic stipling in 22 no lead crystal glass peripheral blood smears but we found 4 basophilic stippling at 22 lead crystal glass peripheral blood smears (Figure 9).



*p<0.05

Figure 9. Morphologic abnormalities of blood cells at peripheral blood smears.

Four peripheral blood smear from lead crystal glasses at 9th, and 10th day revealed basophilic stippling of red cells. These basophilic stipling was shown 18% of total peripheral blood smear from lead crystal glasses. We investigated some changes at 6th, 7th, and 8th days peripheral blood smear from lead crystal glasses, but these imaging changes was unclear for basophilic stippling. I think, these imaging changes was polychromasia. Therefore, we didn't consider these imaging changes. There was statistically significant difference between lead crystal glass and no lead crystal glass with basophilic stippling (Table 2 and 3).

Table 2. Summary of 10-day lead and no lead crystal glass peripheral blood smears imaging results for basophilic stippling. Each day has two peripheral blood smears for lead and no lead crystal glass groups.

Group	0^{th} day	1th day	2th day	3th day	4^{th} day	5^{th} day	6^{th} day	7^{th} day	8^{th} day	9^{th} day	10^{th} day
Lead crystal glasses	–	–	–	–	–	–	**Unclear 2**	**Unclear 2**	**Unclear 2**	**2***	**2***
No Lead crystal glasses	–	–	–	–	–	–	–	–	–	–	–

*p<0.05

Table 3. Percentage change of peripheral blood smears in the microscopic imaging

	No Changes	Unclear Changes	Basophilic Stippling
Leda crystal glasses (n=22)	12 54%	6* 27%	4* 18%
No leda crystal glasses (n=22)	22* 100%	0 0%	0 0%

*p<0.05

X-ray image picture of the four objects was shown in Figure 10. This figure also confirms the availability of lead in either blood or water. Lead containing material appears white in the X-ray images. This image provides a cross-confirmation to the atomic absorption procedure.

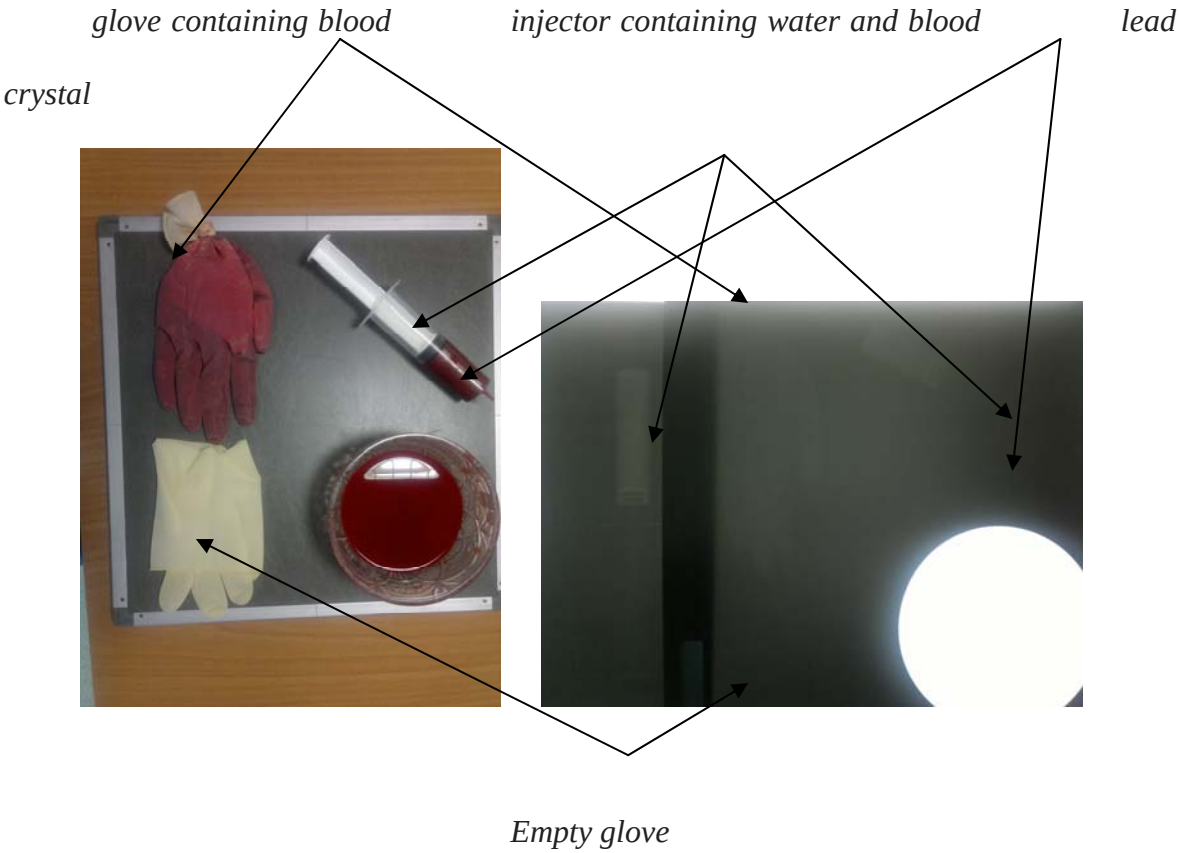


Figure 10. Photografic and X-ray imaging of test material

DISCUSSION

Our findings support the concentration that the lead crystal glass could have suffered lead poisoning from liquid stored in lead crystal glasses. Blood and water stored in the lead crystal glass used in this study reached concentrations as high as 1832 mcg/l and 1653 mcg/l after 10 days of stroge, and in a used, no lead crystal glass reached as low as inconspicuous level at 10 days (Table 1). These results confirm the hypothesis of lead leaching into liquids that are stored in lead crystal glasses.

We prewash and decontamination the glass, residual surface powder from manufacture could not have accounted for the higher rates of lead contamination seen in the first versus the other following days.

We chose to store the liquids at room temperature. If we warmed our liquids, it could have increased the extent of leaching. An experiment by Hoffmann, in which he boiled various wines in leaden vessels according to ancient Roman instruction, found levels of 390-781 mg lead per liter of wine (15).

The rate of lead leaching into liquid has previously been shown to be enhanced with increasing acidity (16). Differences in rates of leaching by two liquids in this sutdy could be explained in their pH, because blood (pH 7.4) had highest lead concentrations, whereas water (pH 6.0) had an intermediate amount of lead leaching.

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15. Hofmann KB. Die Getranke der Griechen und Romer vom hygienische Standpunkte. Arc Gesch Med 1883; 6:26-40.

16. Lin SW, Vargas-Galarza Z, Felix-Navarro RM. Optimizing the conditions for leaching lead from solid waste produced by purometallurgical process of recycling automobile used batteries. J Mex Chem Soc 2006; 50 (2): 64-70

The practice of storing water and blood in lead crystal glasses for variable periods of time before its consumption would increase the risk of lead poisoning. This study showed that stored liquid continuedto leach increasing amounts of lead over time, at least for the first 10 days (Graphic 1). Amount of leaching lead increase proportionally with time. This finding also confirms my first hypothesis.

Lead impacts many organ system, but this study will focus on hematological effects. Lead has no biological function: however, low levels of human exposure have a number of negative consequences such as impairment of the function of renal tubular cells, inhibition of sperm formation, slowing of motor nerve velocity, dysfunction of central nervous system and cardiovascular diseases (17) On the other hand, at high levels there is damage to almost all organs, and most important to the central nervous system, kidneys and blood, culminating in death at excessive levels. Despite the number of reports about lead toxicity (18), very little has been informed about its effects on cell membranes in general and particularly on that of the human erythrocyte.

The hemotological effects are mainly interference with heme and hemoglobin synthesis, and changes of erytrocyte morphology and survival result in the anemia frequently observed in lead poisoning (19).

In my study I showed the toxic effects of erythrocytes on light microscobe. I found the basophilic stippling in the peripheral blood smears (Figure 8). These are important findings that are accepted as some findings of lead poisining. These findings were also similar to the other studies that investigated toxic effects of lead over blood.

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17. Suwalsky M, Norris B, Villena F, Cuevas F, Sotomayor P, Zatta P. Aluminum fluoride affects the structure and functions of cell membranes. Food Chem Toxicol. 2004 Jun;42(6):925-33.
18. Suwalsky M, Norris B, Villena F, Cuevas F, Sotomayor P, Zatta P. Aluminum fluoride affects the structure and functions of cell membranes. Food Chem Toxicol. 2004 Jun;42(6):925-33.
19. Suwalsky M, Norris B, Villena F, Cuevas F, Sotomayor P, Zatta P. Aluminum fluoride affects the structure and functions of cell membranes. Food Chem Toxicol. 2004 Jun;42(6):925-33.

Lead has been found to migrate from lead crystal glass into beverages (20). This problem is especially severe if beverages are stored in lead-crystal containers, e.g., decanters or liquor bottles. This phenomenon was not observed with borosilicate glass containers (21). These findings were also similar our study (Gaphic 1, Figure 9,10)

Kutbi et al. (22) studied 200 boys aged 6-8 years. The pattern of haematological parameters was described as predictive of microcytic anaemia. The finding of this study also is consistent with my study.

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20, Graziano, P. "Lead exposure from lead crystal". The Lancet (1991). 337 (8734): 141-143.
21. De Leacy EA Lead-crystal decanters - a health risk? Med J Aust, 1987 147: 622.
22. Kutbi II, Ahmed M, & Saber A. Measurement of blood-lead levels in school children of Jeddah Saudi Arabia and assessment of sub-toxic levels of lead on some sensitive hematological parameters. J Environ, 1989, Sci Health, A24: 943-955.

CONCLUSION

The original test using blood and water leaching solution was developed to test for lead migration from crystal containing added lead. This work clearly demonstrates that it is also an excellent leaching agent for assessing the safety of crystal containing added lead. The concentration of elements leached water used were all considerably less than the concentrations leached by blood. Therefore, if the concentrations leached into blood are within acceptable limits, it may be safely assumed that stemware manufactured from the glass composition is safe for human use (23). But the study of peripheral blood smear showed basophilic stipplings. Basophilic stippling is equal to anemia and anemia is a harmful state for human beings.

Although we found that significant amount of lead leach into the blood and water from lead crystal glasses, this is an experimental design and we usually never drink water that were waited for 10 days from any glasses. With daily usage style of these glasses, we usually do not expect to have lead intoxication. However we should be aware of the danger. So while using lead crystal glasses, we should give care not keep beverages for longer periods.

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Limitation of this is simply its'design. Although this study is performed over blood as a biological material, this study is an in-vitro study. Therefore the evidence level of this study is not so high. In order to increase this evidence level, a new study should be designed in an in-vivo style (i.e. animal study). By this methodology the real biological effect of the lead intoxication can be observed over living tissues. Furthermore, in order to give a new perspective to this study the effect of lead over the stem cells can be researched. This may be done in two spectrum. One part of this is to study the effect of lead over blood stem cells. In my opinion the second and the most important part is to study over pregnant animals and to see the effect over embrios as the most important stem cell.

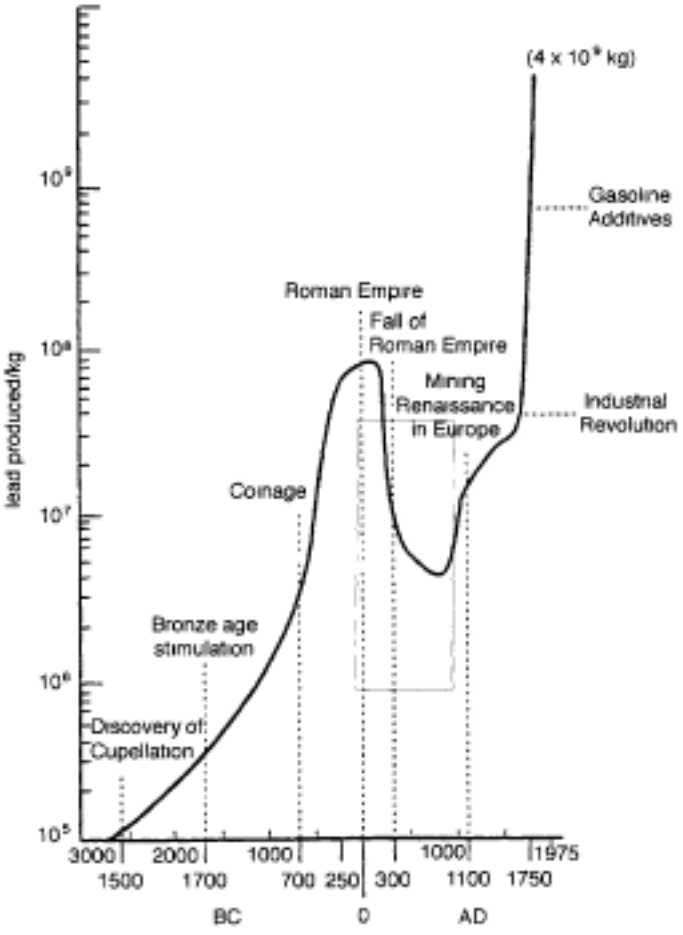
Acknowledgement

I want to thank to Asist. Prof. Suat Doganci, MD (Department of Cardiovascular Surgery), Assoc. Prof. Oral Nevruz, MD (Department of Hematology), Asist. Prof. Ayse Eken, MSci (Deparment of Pharmacology) , Sati Uludogan (Department of Radiology), Assoc. Prof. Ismail Avci, MD (Department of Blood Bank) for their valuable contribution in every step of my study.

APPENDIX

Appendix 1: Use of lead during the past 5000 years.

In medieval and early modern Europe lead glass was used as a base in coloured glasses, specifically in mosaic tesserae, enamels, stained-glass painting, and bijouterie, where it was used to imitate precious stones. The 12–13th century Heraclius details the manufacture of lead enamel and its use for window painting in his De Coloribus et artibus Romanorum (Of for Huereds and Crafts Romans'). This refers to lead glass as "Jewish glass", perhaps indicating its transmission to Europe. The development of lead glass continues through the twentieth century. Lead-crystal continues to be used in industrial and decorative applications.

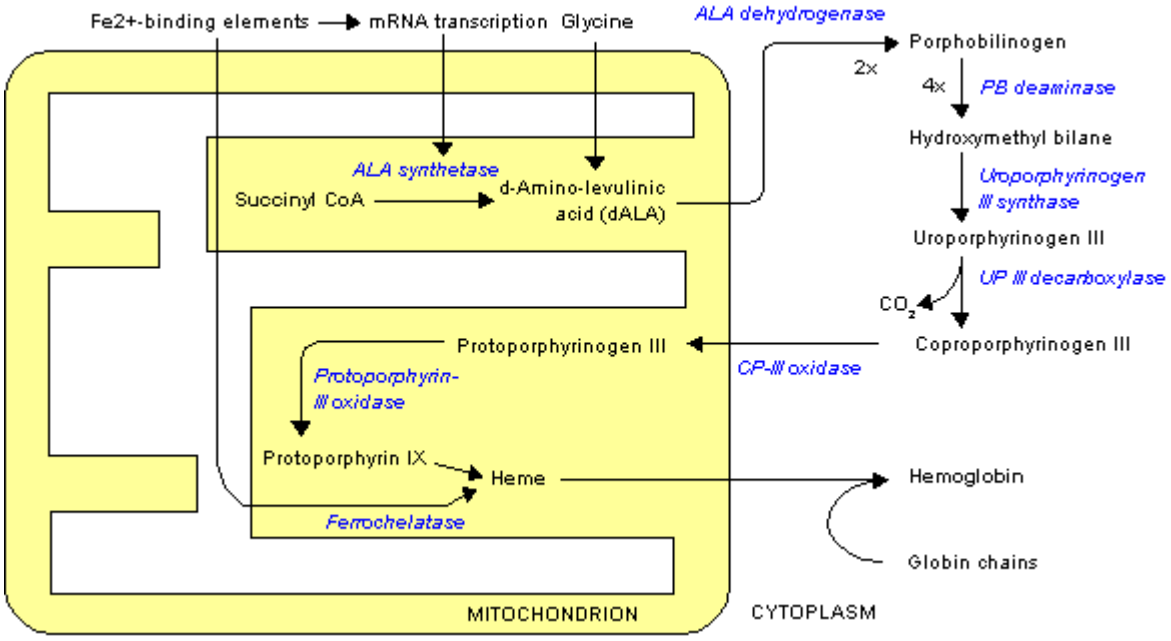


Use of lead during the past 5000 years

Appendix 2: Lead interaction in the heme pathway

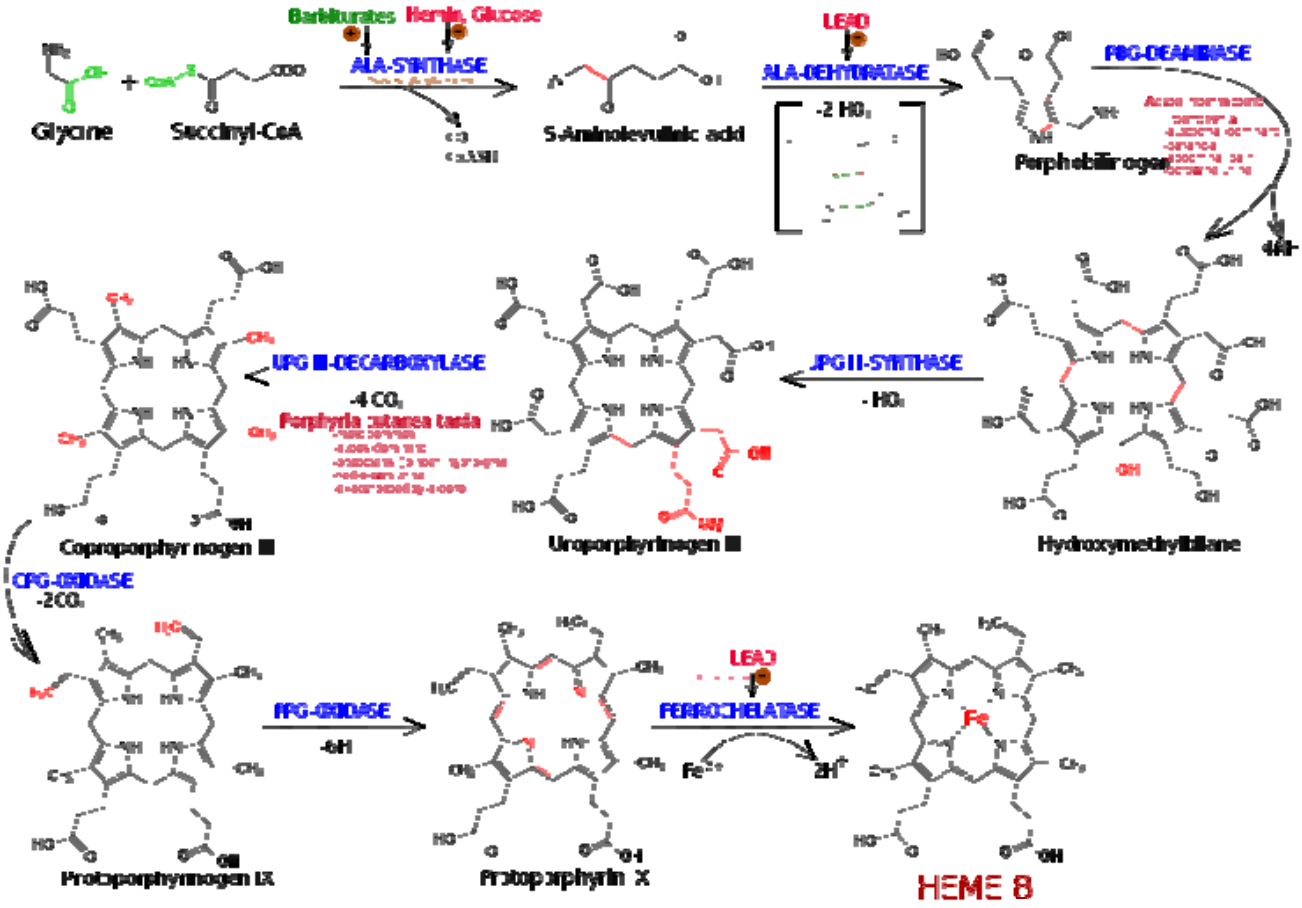
A.

Lead blocks enzymatic action of ALA-Dehydrogenase and ferrochelatase, halting pathway heme synthesis pathway and leading to ALA secretion (in urine) from the body



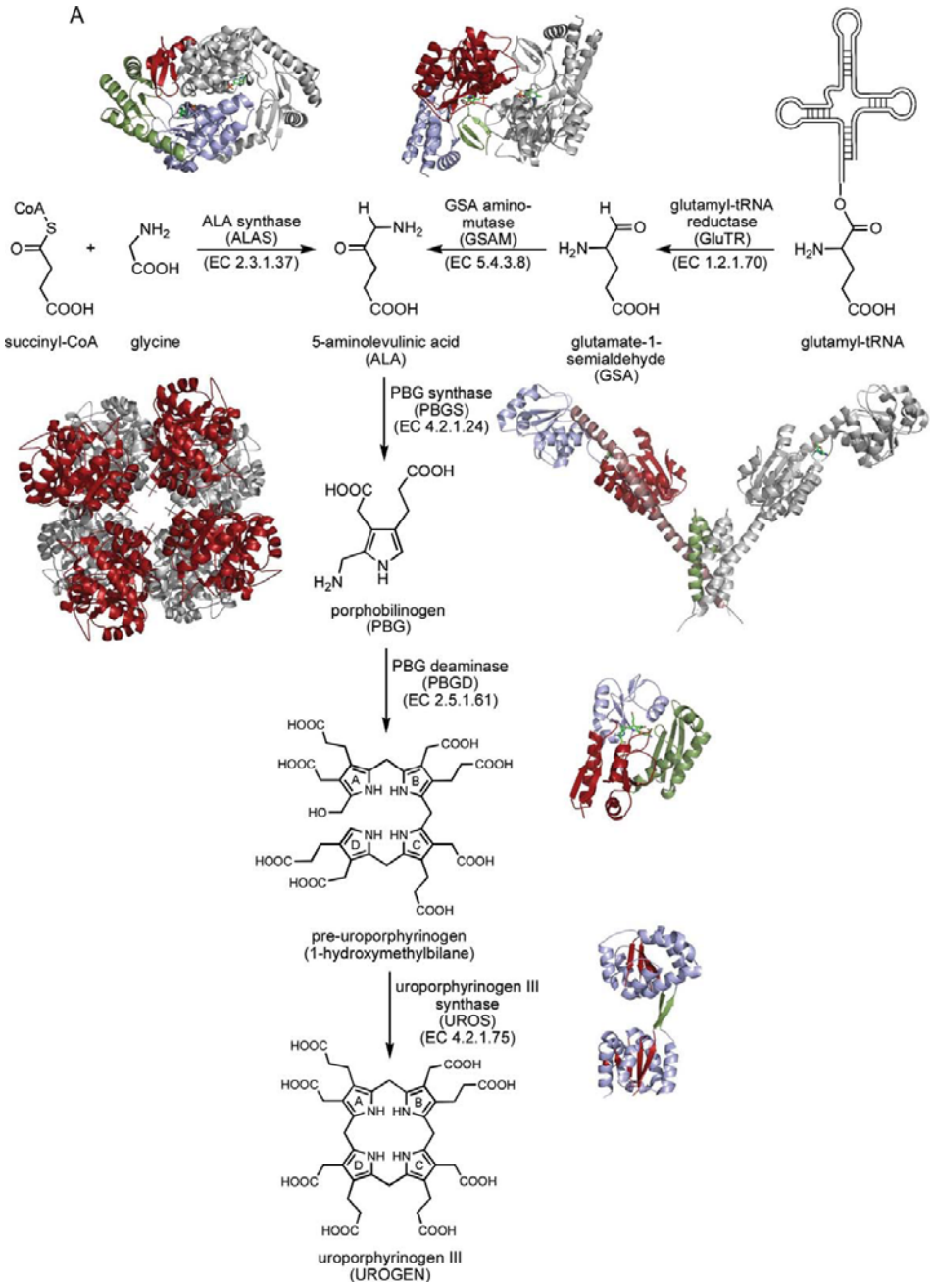
Heme synthesis-some reactions occur in the cytoplasm and some in the mitochondrion.

B.

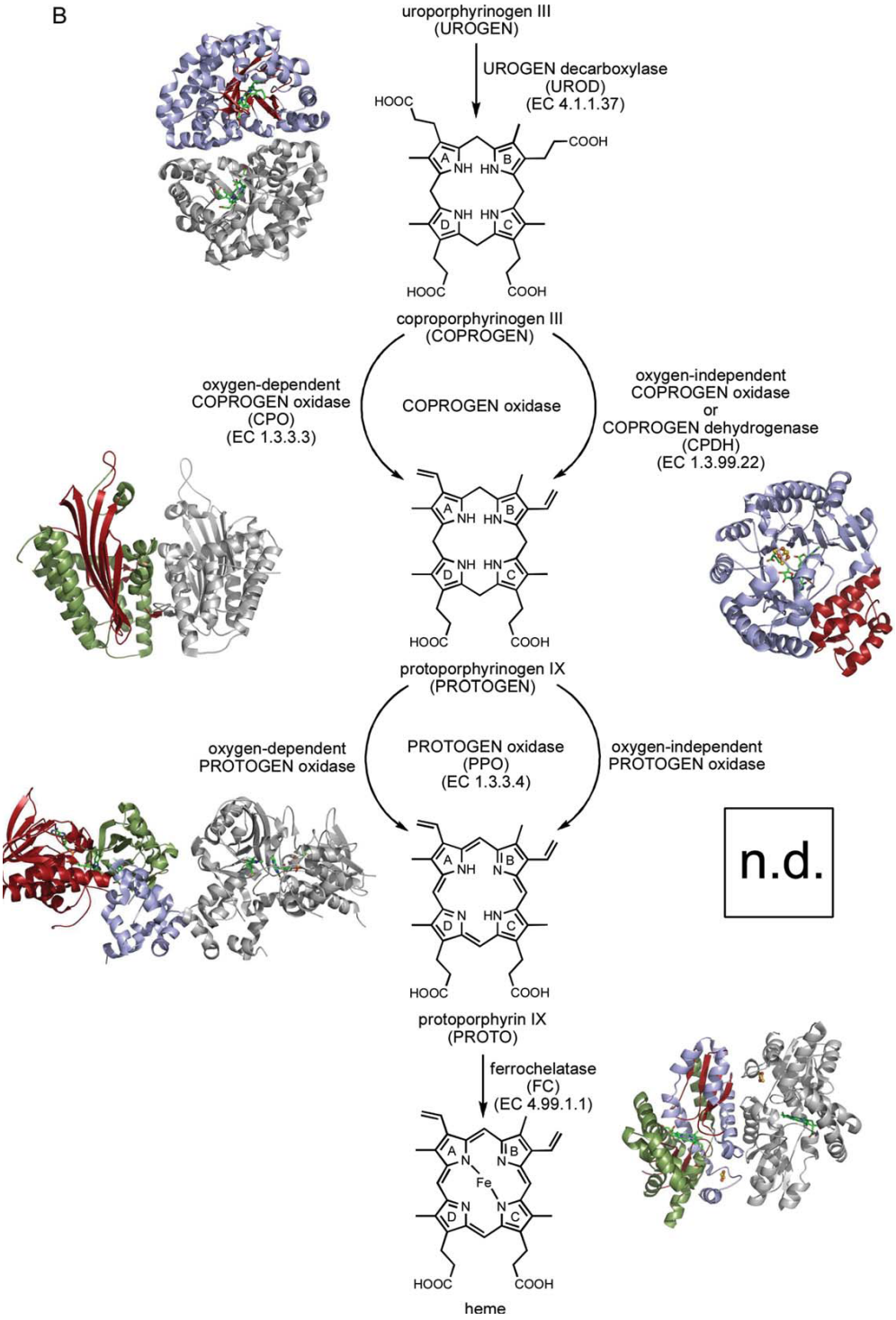


Heme B biosynthesis patway and its modulators. Major enzyme deficiences are also shown here

Appendix 3: Steps of heme biosynthesis.

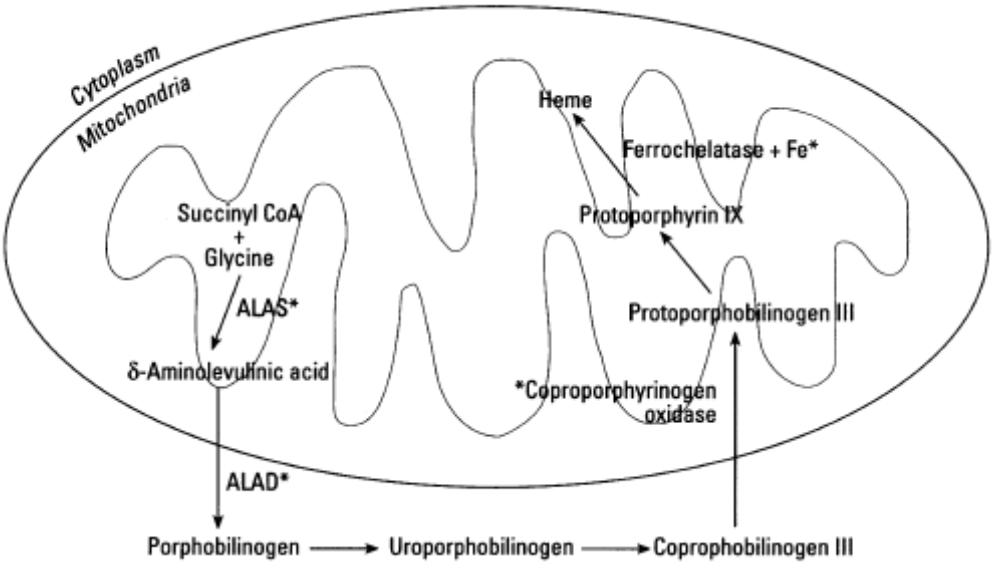


A: The first cyclic tetrapyrrole uroporphyrinogen III is formed from the precursor 5-aminolevulinic acid in three enzymatic steps via the intermediates porphobilinogen and pre-uroporphyrinogen. Depending on the organism, ALA is either synthesized by condensation of glycine with succinyl-CoA or from tRNA-bound glutamate via glutamate-1-semialdehyde.



B: Uroporphyrinogen III is converted into heme in four consecutive enzymatic steps via the intermediates coproporphyrinogen III, protoporphyrinogen IX, and protoporphyrin IX. Structures of all heme biosynthesis enzymes have been determined with the exception of oxygen-independent PPO (n.d., structure not determined).

Appendix 4: Inhibition points of heme by lead.



Lead interactions in heme pathway. ALAS, δ-aminolevunic acid synthase; CoA, coenzyme A. The heme biosynthesis patway is represented. Several enzymes in patway can be affected by lead; two of the most clinically important are ALAD and ferrochelatase. Both these enzymes are inhibited by lead. Their activity can be measured directly or by the measurement of accumulation of their respective subsrates. In the presence of lead, δ-aminolevunic acid accumulates when ALAD is inhibited. Inhibition of ferrochelatase results in the increased production of zinc protoporphyrin.

Appendix 5: Decontamination process

The glasses were kept in a climate chamber for 1 week at a temperature of 50^{o}C and with a relative humidity of 100%. The climate chamber was an isolated box thermostatically controlled with a fan for air circulation. The glasses were placed upside down with a slight tilt. The glasses were rearranged at regular intervals during the treatment. Before and after the treatment, the glasses were washed once in a dishwasher. These glasses were then leached in the same way as the untreated samples.

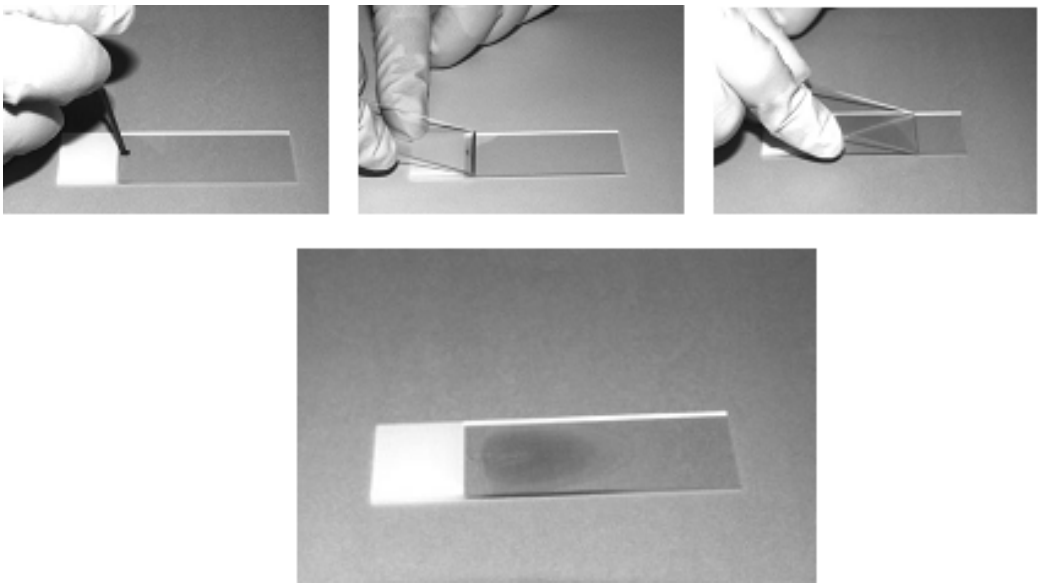
Appendix 6: Lead level testing

Lead concentration was measured by atomic absorption spectroscopy (AAS) (atomic absorption spectrophotometer model A analyst 600 and model A analyst 800 equipped with graphite furnace, Zeeman background correction system, and lead hollow cathode lamp; Perkin Elmer, Norwalk, CT, USA). Six micro liters of the sample mixture prepared with matrix modifier (see below) was heated in a graphite furnace to 2,450°C. During the process, the atomized lead sample was excited for absorbance at λ = 283.3 nm for 2 s by a lead hollow cathode lamp. Matrix-specific standards containing 0,10, 50, 250, and 500 µg/L of lead were prepared by spiking aqueous lead standard solution of 1,000 µg/mL (Perkin Elmer) into water, and blood, respectively. One hundred microliters of standard was mixed with 200 µL lead matrix modifier (2 g ammonium phosphate monobasic in 10 mL 10% Triton-X QS 200 mL H_2O) prior to heating in the atomic absorption spectrophotometer. Each set of standards (0, 10, 50, 250, and 500 µg/L) was used to calibrate for the respective matrix (e.g., standards made in water were used to generate the standard curve for water specimens). Three levels of QC prepared for routine blood lead measurements (two purchased from BioRad, Hercules, CA, USA; one made in lab) were run after calibrations to verify calibrations. The precision (coefficients of variation) for these quality control levels at 80, 300, and 400 µg/L were 4.4, 3.3, and 3.7% (n =200 each), respectively. Experimental specimens (water, and blood) taken at different time points were diluted with the respective blank solution and mixed with lead measured concentrations were within the reportable range of our assay (0–500 µg/L). The laboratory at Department of Pharmaceutical Toxicology, Gulhane Military Academy of Medicine, Ankara, Turkey

Appendix 7: Glass leaching standardization

The all glasses were tested to determine whether similar amounts of lead would be leached from the vessel using the Center for Disease Control (CDC) standardized protocol for lead contamination assessment. Each vessel was rinsed with de-ionized water three times before the addition of 500 mL of acetic acid (4%, v/v). After 24 h, lead content was measured using a similar method as outlined above except that standards were prepared using acetic acid to generate the calibration curve.

Appendix 8: Prepare of a peripheral blood smear and Morphologic examination



Prepare of a peripheral blood smear

A peripheral blood smear is a thin layer of blood smeared on a microscope slide and then stained in such a way to allow the various blood cells to be examined microscopically. Blood films are usually examined to investigate hematological problems. Blood films are made by placing a drop of blood on one end of a slide, and using a spreader slide to disperse the blood over the slide's length. The aim is to get a region where the cells are spaced far enough apart to be counted and differentiated.

The slide is left to air dry, after which the blood is fixed to the slide by immersing it briefly in methanol. The fixative is essential for good staining and presentation of cellular detail. After fixation, the slide is stained to distinguish the cells from each other.

Morphologic abnormalities of blood cells were discovered by microscopic examination with the oil immersion lens of well-prepared films of peripheral blood stained with Wright's stain. For appropriate interpretation of the morphology of erythrocytes, one concentrates on areas of the slide where the red cells appear singly and have central pallor. Examination of erythrocytes far out on the feathered edge discloses erythrocytes lacking central pallor, whereas in thick areas of the slide the morphology of the erythrocytes was distorted by contact between cells. Hematoloic examination was performed in Department of Hematology, Gülhane Military Medical Academy, Ankara, Turkey.

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