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X-RAY FLUORESCENCE ANALYSIS STUDY
FINAL REPORT

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Co-Investigator: G. Laurer, Ph.D.

12/1/70 - 12/31/77

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ESB

FINAL REPORT
to the
DEPARTMENT OF ENERGY

Title of Project:	X-Ray Fluorescence Analysis Study
Period Covered by Report:	December 1, 1970 - December 31, 1977
Principal Investigator:	Theodore J. Kneip, Ph.D.
Co-Investigator:	Gerard R. Laurer, Ph.D.
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MEASUREMENT OF LEAD IN BLOOD UTILIZING RADIOISOTOPE-
EXCITED X-RAY FLUORESCENCE ANALYSIS

I. Introduction

The high incidence of lead poisoning in young children (1-6 years) living in old, deteriorating housing remains a major public health problem. It has been shown that lead-based paint, the most common source from which these children absorb toxic amounts of this material, is ingested in the form of flakes or peelings from damaged and unrepaired surfaces covered with paint which may contain large amounts of lead. A study of New York City tenement apartments indicated, at a significance level of 0.05, that greater than 90% of these apartments have at least one surface covered with paint containing $> 1.0 \text{ mg/cm}^2$ lead (1). The significance of this number is realized when one considers that the suggested Daily Permissible Intake (DPI) of $200 \text{ } \mu\text{g}$ (2) for a child of 2 to 3 years old may be obtained if he ingests approximately one square inch/day of paint containing 0.03 mg/cm^2 of lead.

Our involvement in this problem began several years ago at the request of the New York City Health Department for a fast, dependable, non-destructive method for determination of lead in paint to aid their growing lead poisoning control program. As a result, a lead paint detector was developed based on the detection of the K X-ray fluorescence using a radioisotope, Cd-109, for excitation. A lithium-drifted, germanium (Ge(Li)) detector was used to measure the lead

X-rays. The prototype detector has been used successfully in a test study of lead paint distribution in New York City (1).

Although the use of this instrument will help lessen the lead paint hazard in city slums, the enormity of the problem (New York City estimates there are some 30,000 tenements, each containing an average of ten apartments in which the risk of childhood lead poisoning is high) indicates that a considerable amount of time will pass before the problem is on the way to being solved. During this time children will continue to be exposed to lead-based paint. Because of this, emphasis has also been placed on developing methodologies for the measurement of lead in blood, especially focusing on the development of screening tests which would allow rapid separation of children into several risk categories. In this manner, those at the highest level of risk could receive immediate treatment while separated from the environment in which they were contaminated and allow, concurrently, decontamination of that environment as well.

One of the most serious difficulties encountered in any procedure for determining lead in blood is contamination, especially when it is desirable to limit the sample size to as small as possible, as it would be when obtaining the sample from a young child. Because of the presence of lead everywhere, it has been found difficult to maintain a screening program based only on the measurement of lead in blood, but rather on the measurement of one of the biochemical metabolites resulting from the presence of lead and its interference with heme synthesis.

Several such products of blocked heme synthesis have been tested - delta-aminolevulinic acid in urine, delta-aminolevulinic acid dehydratase in erythrocytes, coproporphyrin in urine -- but the one that seems to be most useful for monitoring the toxic effects of lead in blood (lead itself is not measured) is the protoporphyrin (FEP) or zinc protoporphyrin (ZPP). Lead blocks the incorporation of iron into protoporphyrin, with the result that this material accumulates in the red cells. A direct and linear relationship has been demonstrated between the lead and erythrocyte protoporphyrin in the circulating blood, and methods based on this finding now are preferred for primary screening (3). The method has two shortcomings, however: (i) children with iron deficiency diseases who have not ingested lead also show increased erythrocyte protoporphyrin from direct lack of iron; and (ii) because of the lifetime of the red cells, the effect of lead on heme synthesis as indicated by increased protoporphyrin does not show up immediately. Thus, an acute episode of lead absorption will not result in an immediate elevation of FEP. This test, therefore, is a useful preliminary screen to blood lead analysis, measuring the toxic effect of lead and confirming the actual measurement of lead in the blood due to chronic ingestion as opposed to a false indication of lead from sample contamination; it cannot substitute, however, for the actual measurement of blood lead. Although it requires a small amount of chemical preparation, the test, including the fluorimeter used to measure the protoporphyrin, is field adaptable.

For the reasons cited above, viz., lead contamination and weaknesses in the porphyrin test, a single screening test, either FEP or blood lead, cannot be used to categorize children for priority follow-up. Consequently, screening procedures recommended by the Center for Disease Control (4) utilize both tests to determine the potential risk of lead poisoning in screened children. To evaluate the relative risk of lead poisoning and the priority for medical evaluation and environmental intervention, children are divided arbitrarily into four classes as indicated in Table I. Class IV children are at urgent risk of lead poisoning and should be provided immediate medical evaluation. As stated in (4), in no case should they be evaluated later than 48 hours after the results of the tests are known: if possible, within 24 hours. Class III children are at high risk, Class II are at moderate risk, and Class I children at low risk.

Measurement of Blood Lead

Blood lead analysis at most health laboratory facilities is usually done by atomic absorption spectrophotometry (AAS), although many are now using a more recently developed method called anodic stripping voltometry (ASV), an electro-chemical procedure.

With the AAS methods, after chemical pretreatment of the blood, the sample is atomized by aspirating it into a flame or injecting it into a carbon-rod (flameless) atomizer where the molecules are dissociated into atomic form. In atomic

TABLE I

Risk Classifications for Asymptomatic Children

Test results of blood lead ($\mu\text{g}/\text{dL}$)	Test results of erythrocyte protoporphyrin			
	≤ 49	50-109	110-249	≥ 250
Not done	I	*	*	*
≤ 29	I	Ia	Ia	EPP+
30-49	Ib	II	III	III
50-69	**	III	III	IV
≥ 70	**	**	IV	IV

EPP+ = Erythropoietic protoporphyria

* = Blood level necessary to evaluate risk

** = Combination of results not generally seen in practice;
if observed, retest with venous blood immediately

Ia = Children with iron deficiency

Ib = Children who appear to have transient, stable, or
declining blood lead levels and are at low risk

absorption spectrophotometry, the beam of a hollow-cathode lamp, radiating at a specific frequency, is passed through the flame and the amount of absorption of the beam is proportional to the concentration of lead in the sample. Either of these techniques, flame or flameless, has more than sufficient sensitivity (1 to 10 $\mu\text{g ml}$ of whole blood = 0.01 to 0.1 ppm) and is capable of measuring well below "normal" concentrations of blood lead.

The same technique originally required large (up to 5 ml) samples, and in very young children this amount of blood generally must be drawn from a vein in the neck. Mothers and doctors alike are reluctant to take a sample unless necessary. Moreover, it is often necessary to take more than one sample. The blood lead concentration measured in the first sample, if high, may be the result of a single, recent ingestion of a large piece of leaded paint, rather than being representative of an equilibrium level established by long-term chronic ingestion. To determine which is the case by blood lead measurement, serial sampling is required, and there is a reluctance to subject the child to this when such large samples are required. Since sound diagnosis and effective treatment planning depend on the establishment of this information, especially when chelation therapy may be necessary, this factor was a severe drawback to the use of these methods.

Other methods have been developed now which eliminate the need for very large blood samples. One uses an additional

piece of apparatus called a Delves cup, attached to an atomic absorption spectrometer (5). Rather than extracting the sample chemically from a large volume of blood, a drop of blood ($\sim 10 \mu\text{l}$) from a lanced finger is collected (i.e., absorbed freely) on filter paper so as to form a spot of not less than 0.24 inch in diameter. After drying a 0.25 inch disk is punched out of the filter directly into the Delves cup, which is then in turn inserted into the flame. The spectrometer output, in the form of spikes on a strip-chart recorder, is proportional to the lead concentration in the sample disk. The problems encountered with this method, as with other micro-methods, is one of sample contamination. Because of the ubiquitousness of lead, the sampling technique, in any event, must be scrupulously clean, and the smaller the sample, the more difficult it will be to control contamination. Surface contamination of the skin in the case of a "finger-stick" sample may be a very significant problem when the amount of blood taken is as small as $10 \mu\text{l}$ (especially for samples drawn in the field. Also, sampling precision may be a problem with the very small sample size (6).

In addition to the FEP test, which was developed since the start of the XRF work, another method for the direct measurement of lead in blood has been under development and is also now being used in laboratories as mentioned earlier, i.e., anodic-stripping voltometry. Using a newly-developed mercury-graphite electrode, ASV is easily capable of measuring "normal" levels of lead in blood. Although it previously

required a perchloric acid digestion step to liberate lead bound to erythrocytes, recent work (7) has described a simplified procedure eliminating the digestion step. A metal-exchange reagent is now used to lyse the red cells and release the bound lead. With this procedure, a 100 μ l blood sample is used. Although the sample pretreatment is now much simpler than required with other methods, the method is also less versatile in terms of the number of elements it is capable of analyzing, a disadvantage if a laboratory must a variety of demands. Nevertheless, with the simplified sample treatment and relatively simple-to-operate instrumentation available for it, the ASV blood-lead method, like the FEP test, is field-adaptable. Once calibrated, the ASV method is said to be capable of performing 30 lead analyses per hour.

In addition to the problems discussed, the above methods require skilled technicians and the use of expensive and delicate apparatus. This has restricted the use of these techniques, therefore, to centrally located laboratory facilities. The blood samples required, whether large or small, are thus generally obtained at a hospital or local health clinic where the children are brought, with or without symptoms of lead poisoning. The sample is then sent to the central laboratory where the analysis for lead is made. The need to send samples to central facilities to be analyzed leads to another problem, i.e., loss of time between sampling and analysis, as well as turn-around time between analysis and notification of parents and/or responsible agencies to

hasten initiation of proper remedial action in the case of a borderline over-exposure. Depending upon sample load, this time lag could be as long as a few weeks in larger cities. The same is true in more rural areas where the local health unit may not have the facilities or the equipment to make the necessary measurements and therefore must also send the sample to the larger city laboratory for analysis. In either case, that time may be critical for some children.

The necessity of serial sample analyses, coupled with the lag in time between sampling, analysis, and notification, has thus focused attention on the need for a system capable of on-the-spot detection and measurement of lead in blood. This system should require a small, easy-to-obtain sample, i.e., one obtainable with a finger-stick but not so small as to make collection contamination a difficult problem; it should be simple to operate, requiring a minimum of training to make a sample measurement; sample preparation should be as short as possible and require a minimum of skill; it should be small, reasonably rugged and transportable, capable of being set up quickly and operated at regional health clinics throughout a city, or a mobile operation in a van, e.g., usable by local health units in the suburban as well as the more rural areas. This would provide a device able to classify children rapidly into the various categories of blood lead concentrations and, in conjunction with the EP test, into the required risk categories. With such a device, programs actively seeking blood samples could be emphasized, in conjunction with wall testing programs.

Our work with the use of X-ray fluorescence (XRF) analysis for the measurement of lead in wall paint indicated with sufficient increase in sensitivity, this technique would allow detection and measurement of sub-ppm levels of lead in blood. Moreover, as with the detection and measurement of lead in wall paint, the use of a radioisotope for X-ray fluorescence excitation would lend itself to making the system more efficiently transportable.

The primary purpose of the work to be described, therefore, has been the development of a system for the detection and measurement of lead in blood utilizing radioisotope excited X-ray fluorescence (XRF) analysis. The goal for practical application is a system which will measure "normal" levels, i.e., $< 0.3 \mu\text{g}/\text{ml}$ (0.3 ppm, 30 $\mu\text{g}\%$), of lead in an amount of blood obtainable with a finger-stick or other sampling method, requiring as little sample preparation as possible, and with as short an analysis time as possible. In addition, the system should be readily transportable, capable of being operated at any location with a minimum of technical effort.

The XRF technique is also capable of analyzing approximately 30 samples per hour. Moreover, as will be shown, it has some distinct advantages and options over other methodologies. In addition to being transportable in the sense of being easily set up and operated at almost any location for blood lead analysis, the system lends itself to operation directly in the field, in a van, or neighborhood clinic, for example,

because of the simplicity of sample preparation which requires only centrifugation and drying of the sample. With the use of the available state-of-the-art, hyperpure germanium detectors, the same detector used for blood analysis may be taken directly into a home or apartment and used to measure lead in wall paint, which would enable rapid identification of the source of lead in the case of very high blood levels. Also, because of the nature of the fluorescence process, the XRF system is capable of quantitative, simultaneous analysis of the other elements in blood, in addition to lead. Thus, the XRF system would provide a further check in those instances where the EP test is positive, either as a result of an iron deficiency caused by lead intoxication or due to an anemic condition from other causes. In either case, the free erythrocyte protoporphyrin being measured in the FEP test contains a zinc atom rather than iron, as it would in normal heme synthesis, and the zinc in the erythrocytes, as well as the iron, may be measured for comparison to normal levels simultaneously with lead.

II. X-Ray Fluorescence Spectrometry

When the atoms in a specimen are bombarded by high energy photons (X rays or gamma rays) or high energy, charged particles (electrons, beta particles, protons or alpha particles) the energy carried by these particles is transferred to the atoms, exciting bound electrons

from their fixed energy levels in the atom. The vacancy thus created in the electron structure of the atom is immediately filled ($< 10^{-15}$ seconds) by another electron from a less energetic level and the energy difference between these two levels is released in the form of an X-ray photon. Because each element has atoms with fixed and specific electron energy levels which are different than all other elements, the X-ray energy released can be used as a fingerprint, to identify the element from which it was emitted. Also, the number of X rays emitted is directly proportional to the number of atoms of the element present in the specimen. Thus, by measuring the energy of the X rays emitted from the specimen and counting the numbers of them that correspond to each element of interest, it is possible to obtain an elemental analysis of the specimen.

The classical method of measuring X-ray energy was to utilize the phenomenon of diffraction to measure, via a "perfect" crystal and a variable detector positioner, the diffraction angle and, with the Bragg equation, the effective wavelength from which the energy was calculated. This "wavelength dispersive" method of analysis has been replaced in many applications by the "energy dispersive" method in which the energy of the X rays are measured directly. This method owes its current popularity to the rapid development

(over the past few years) of silicon and germanium detectors capable of resolving the X rays from adjacent elements in the periodic table.

Because of the precise geometrical relationships involved in wavelength dispersive techniques, well-collimated X-ray beams are required and hence only a small solid angle of the X rays emitted from the sample in all directions may be used. Since this degree of collimation is not required in energy dispersive analysis, this technique is inherently much more efficient. Coupling this with its ability to measure all energies, and hence all the elements present, simultaneously, makes the energy dispersive technique most useful for the elemental analysis of blood.

III. Application of Technique to Blood Lead Analysis

A. Materials and Methods:

In contrast to other applications of X-ray fluorescence analysis for elemental assay, in which the goal is multi-element analysis, the detection and measurement of normal levels of lead in blood requires optimum excitation and resolution of the characteristic K or L shell X-rays of that element from those of the other elements present in the

sample matrix when all are excited simultaneously. Optimum excitation, as seen in the previous section, is obtained when the incident energy is above, but close to, the K or L shell absorption edge energy of the element to be fluoresced. Other elements with absorption energies below this also will be excited, but not as efficiently. For the field operation under consideration, the choice of mode of excitation is limited for practical reasons to photons, which may be obtained from X-ray tubes or radioisotope sources. Both of these have advantages and disadvantages which will be discussed below.

Resolution of the lead X-rays from those of other elements in the blood sample is dependent in part on the selection of the fluorescence X-ray energies for detection, i.e., K or L shell. Lead is the only element of high atomic number ($Z=82$) present in blood at concentrations of $0.1 \mu\text{g/gm}$ or greater, and since matrix absorption of its 75 keV and 85 keV K_{α} and K_{β} X-rays would be negligible in the small samples anticipated, excitation of the K shell of lead should offer an interference-free spectral region for measurement and make the resolution problem relatively unimportant.

The Compton scatter due to the sample matrix when using the 88 or 122 keV gamma rays of Cd-109 or Co-57, the most efficient lead K shell excitation sources, can cause interference at those energies due to the broadness of back-scattered peak which tends to overlap the 75 keV K_{α} peak of lead. Also, because of the height of the scatter peak relative to the fluorescence peak at small concentrations, this

interference is magnified in the case of small sample sizes. Examination of the absorption coefficients at the K and L shells' critical-absorption edges as shown in Figure 1, indicates an increase of approximately 20 in absorption probability at the L shell edge energies compared to the K shell. Accounting for the difference in fluorescence yield* ($\omega_L = 0.40$), for equivalent numbers of excitation photons, the L shell represents a possible net gain of approximately 10 in X-ray output, thus indicating that our sub-ppm measurement goal would be better achieved by using the L X-rays of lead. In this case, resolution of the lead L X-rays from the K shell X-rays of other elements present, as well as matrix scatter of the source, is necessary.

1. Detector Selection: A lithium-drifted, silicon (Si(Li)) detector is the optimum X-ray detector for detection of the L X-rays because it provides both the required resolution and the needed intrinsic efficiency. The full energy peak efficiency versus energy for typical silicon and germanium detectors is shown in Figure 2. Below approximately 16 keV a 3 mm thick Si(Li) detector is for practical purposes 100% efficient. The most abundant L shell X-rays of lead are all below this, at energies of 14.762 keV (L_Y), 12.620 keV (L_{β_2}), 12.611 keV (L_{β_1}), 10.549 keV (L_{α_1}), and

*Fluorescence yield is the ratio of the number of X-rays emitted to the number of electron vacancies produced.

10.448 keV (L_{α_2}). Since the detector must be housed in a vacuum tight container, it is necessary to provide an X-ray entrance window. Also shown on the right-hand portion of Figure 2 are curves of X-ray transmission versus energy for several thicknesses of beryllium. A 5 mil thick beryllium window was selected for 100% transmission above 10 keV. In order to retain its superior resolution capabilities, it is necessary to cool the Si(Li) detector with liquid nitrogen. This increases the size and weight of the system because of the need for a cryostat and liquid nitrogen dewar, which also require extra care and maintenance. Nevertheless, these requirements should not detract from its usefulness as a screening device. Although a 30 liter dewar was selected for the development work because of the convenience of the approximately three-week liquid nitrogen holding time, smaller dewars with a sufficient volume for one week or even one day are available, thus making the size and weight of the detection system tailorable to specific requirements.

Selection of the Si(Li) detector size was based on two factors, resolution and geometrical efficiency. Resolution should be good enough to separate one, if not both, of the most abundant L X-rays of lead (10.5 keV and 12.6 keV) from the X-rays expected from other elements in the blood, and the detector area should be as large as possible to obtain maximum geometrical efficiency. Since the resolution obtainable from a Si(Li) detector is an inverse function of its size, the choice was necessarily a compromise, dictated primarily by the

other interfering X-rays to be expected from blood. Table II is a listing of the normal values, with ranges, of the electrolyte content of human blood (8). Also listed are their characteristic X-ray energies.

While there are a great number of elements in blood and all will contribute to matrix effects in the sample, it is noted that in terms of excitation and detection of Pb L α -rays, Rb and Br with K_{α} and/or K_{β} energies above and near 13.044 keV, the Pb L_{III} absorption edge, are the elements that may cause secondary fluorescence of Pb; the others (Au, I, Hg, Ag, Sn) and K_{α} , K_{β} energies considerably higher than the $L_{I,II,III}$ edges, and in addition are present in very low concentrations. All of the elements will, of course, act as absorbers of the Pb X-rays, especially Cl, P, K, Na, and Fe, because of their high concentrations. Rb ($\sim 800 \mu\text{g}/100 \text{ ml}$), and Zn ($\sim 900 \mu\text{g}/100 \text{ ml}$) are the elements to be accounted for in terms of their possible spectral interference with the lead peaks. This is due to the concentrations present, and the proximity of their K-shell X-ray energies to the L X-rays of lead. The K_{α} and K_{β} of bromine, at 11.9 keV and 13.4 keV, and the 15.1 keV and 13.35 keV K_{β} and K_{α} peaks of rubidium (Table I) are thus seen to bracket the 14.7 keV L_{γ} and 12.6 keV L_{β} X-rays of lead, while the K_{α} X-ray of bromine (11.9 keV) and the 9.6 keV K_{β} X-ray of zinc bracket the 10.5 keV L_{α} X-ray of lead.

TABLE II
Elemental Content of Whole Blood

<u>Element</u>	<u>Kα, Kβ X-ray Energy (keV)*</u>	<u>Whole Blood Value (μg/100 ml)</u>
Al	1.48, 1.53	(21-94)
Br	11.91, 13.29	810 (330-1730)
Ca	3.69, 4.01	9,700
Cl (Chloride)	2.62, 2.82	295,000
Cr	5.41, 5.94	3.5
Cu	8.04, 8.91	98 (72-124)
Fl	0.68, -	(11- 45)
Au	9.7, 11.5*	1.2
I (Total)	3.9, 4.3*	9.7 (2.5-16.9)
Fe	6.40, 7.06	(45,500-52,300)
Pb	10.5, 12.6*	29 (18-49)
Li	0.052, -	1.9 (0.9-3.0)
Mn	5.89, 6.49	4,040 (3580-4500)
Hg	9.98, 11.90	0.385 (0.16-0.51)
Pb (total)	2.01, 2.14	35,000
K	3.31, 3.59	168,000
Rb	13.59, 14.96	303 (260-345)
Si	1.74, 1.83	830
Ag	2.98, 3.15*	trace
Na	1.04, 1.07	200,000
S (total)	2.31, 2.46	1,928
Sn	3.4, 3.6*	13
Zn	8.64, 9.57	880 (480-1280)

*L α , L β X-ray energies.

Due to its low abundance and proximity to the K_{β} X-ray of rubidium, the L_{γ} X-ray of lead was dismissed as a possibility for analysis. Attention was focused on the remaining L_{α} (10.5 keV) and L_{β} (12.6 keV) X-rays of lead, both of which are separated by approximately 900 eV from the possible interfering X-rays. On the basis of this 900 eV spread, it was decided that the minimum resolution that could be tolerated at these energies would be approximately 350 eV, fwhm (full width of peak at $\frac{1}{2}$ maximum height). Literature on commercially marketed detector systems indicated that this resolution would be attainable with a 200 mm² Si(Li) detector available with guaranteed resolutions of < 300 eV, fwhm, at the 5.9 keV Mn K_{α} energy. The system eventually obtained* for this project had a guaranteed resolution of 270 eV, fwhm (5.9 keV) and checked in our laboratory, using the 6.4 keV Fe K_{α} X-ray, at 280 eV, fwhm. Another detector was subsequently obtained with a resolution of 170 eV and an area of 80 mm².

2. Source Selection: One of the most important considerations in the use of the X-ray fluorescence technique as mentioned earlier is the selection of the proper excitation source. X-ray tubes, because of their large X-ray flux output, are important in wavelength dispersive systems because of the inherently low efficiency of this method. However, due to

*Model # 7016-16270, Ortec, Inc., Oak Ridge, Tennessee.

the bremsstrahlung radiation content of their beams, X-ray tubes, at least of the high power variety, are not in common use as excitation sources in most laboratories utilizing energy dispersive analysis. Several systems are now available which use specially-designed, low-power tubes with transmission targets as energy dispersive sources. Their power as requirements and size due to this factor still preclude their use when considering a source for a portable, field-type operation. The majority of field applications using the energy dispersive method of X-ray fluorescence analysis (9) utilize a radioisotope source of X-rays or gamma-rays as the exciter because of their portability, low cost, and reliability.

Radioisotope sources for X-ray excitation also have limitations, of course. For safety, sources are generally kept to millicurie strengths, thus limiting the available X-ray or gamma-ray output to the order of 10^7 to 10^8 photons/sec. Also, depending on the application, it is often very difficult to obtain an isotopic source which: 1) has the correct energy X-rays or gamma-rays for efficient excitation; 2) is free of other interfering radiations; and 3) has a reasonably long half-life. Despite these limitations, it is expected that radioisotope excitation sources will continue to find more applications in X-ray fluorescence analysis because, unlike X-ray tubes, they are relatively easily molded into a variety of configurations affording increases in geometrical efficiency which can offset their output deficiencies.

The most efficiency production of characteristic X-rays arises from absorption of photons with energies just above the corresponding absorption edges. Commercially available isotopes include several with X-rays in the energy range of the L absorption edges of lead, viz., Cd-109, Pu-238, and Am-241. Cadmium-109 ($t_{1/2} = 453$ days) decays by electron capture to Ag-109m, which decays with the emission of the 22-25 keV Ag K X-rays (100% abundant) and a gamma-ray of 88 keV (3.6% abundant). Plutonium-238 decays by alpha particle emission with a half-life of 86 years, and the U-234 daughter emits L X-rays of 11.6 keV, 13.5 keV, 16.4 keV, 17.2 keV and 20.2 keV in approximately 13% of its disintegrations. Americium-241 decays by alpha particle emission also and has a 433 year half-time. The Np L X-rays are emitted in the range of 11.9 keV to 22.3 keV in 40% and a 59.5 keV gamma-ray in 35% of the disintegrations. The difficulty of shielding against the 59.5 keV gamma-ray ruled against the use of this isotope despite the relatively high abundance of the Np X-rays compared to the U X-rays of Pu-238 decay.

The relatively short half-life of Cd-109 would involve replenishing or replacing the source rather frequently in order to keep the detection limit stable with short counting times. For operation in the field, this would be inconvenient. The relatively high abundance of the 88 keV gamma-ray could cause background problems due to scattering as well as shielding problems in the design of the source. Finally, the

cost of Cd-109, at least the isotopically pure, accelerator-grade material, is considerably greater than the cost of Pu-238. For these reasons, Pu-238 was chosen as source for the blood lead problem. The compromise between half-life, cost and X-ray abundance between the two isotopes is partially offset by the fact that the uranium L X-rays are closer to the L absorption edges of lead, increasing the absorption of these X-rays by about 2.5 times over those of Cd-109.

3. Source-Sample-Detector Geometry: The limitation of the radioactive source to the tens of millicurie range limits the available X-ray output for excitation to the order of 10^7 to 10^8 photons/sec as opposed to an X-ray tube output of 10^{10} to 10^{12} photons/sec. Once the radioisotopic source and the detector have been selected, therefore, the criterion of a detection limit in the sub-ppm range must be met in the overall design. More specifically, to obtain the lowest possible detection limit with a given X-ray flux, the source design (source holder and collimator) must optimize the source-sample-detector geometry. In essence, this means placing the source as close as possible to the sample to maximize detection efficiency. To realize fully the benefits of fulfilling the above design requirements, the source-sample-detector arrangement should also: 1) minimize the number of directly transmitted X-rays from the source to the detector; 2) minimize the scatter and fluorescence from

structural materials around the source, including the instrument itself; 3) maximize the fluorescence to scatter ratio from the sample.

The flexibility realizable in the structure and encapsulation of radioisotope sources of radiation has led to a number of different source-sample-detector arrangements for various applications of the X-ray fluorescence technique, all of which have taken into account to a greater or lesser degree the above requirements. In his review of the state of development and applications of X-ray emission spectrometry using radioisotope X-ray and gamma-ray sources for excitation, Rhodes (9) discusses a number of the typical source-sample-detector arrangements now in use. For purposes of this report, however, and to discuss the genesis of the source design for the blood lead problem, it will be sufficient to mention only the two most generally used configurations, the central source and the annular source.

Typical arrangements of these are shown in Figure 3. Central source geometry is the most efficient for large detector windows and has found the widest application with scintillation and proportional counters where the geometrical efficiencies for a two-inch diameter detector window, a two-inch diameter sample, and a 1/2-inch diameter source approaches ten percent. However, for small detectors such as Si(Li) where a usable surface area of 200 mm² means of diameter of

only 16 mm, this geometry is impractical because of the loss of counting efficiency due to the "shadow" effect of the central source position.

A form of the annular source configuration is generally used for small detector windows. This configuration is not only desirable for small detectors, but is particularly useful when the low specific activity of the source necessitates a large source area. Our early attempts at source holder design were directed at obtaining a modification of the annular source configuration which would allow the maximum geometrical efficiency while maintaining the other criteria mentioned earlier concerning direct transmission, scatter and fluorescence from structural materials, and sample fluorescence to scatter ratio.

As seen in Figure 3, a disturbing feature of both central source and annular source configurations is the distance between sample and detector when the source is maintained between the sample and detector. Generally, the requirements of flux on the sample plus avoidance of direct detector irradiation have made this a relatively large distance with the resulting decrease in sample-detector geometry. Geometrical analyses made on variations of the annular source configuration in which the source axis with respect to the detector axis was varied between zero and ninety degrees, to allow the sample to be moved closer to the detector, showed no substantial improvement. As the

source axis is tilted, allowing the sample to move closer to the detector, the source must be more stringently collimated and moved further from the sample to avoid direct transmission of the excitation flux to the detector, thus in effect reducing source to sample geometry. Also, as the source angle approaches 90° , background counts from scatter and fluorescence of the source structural materials would become large.

Since the design for blood lead analysis is directed only at the detection and measurement of the lead X-rays, unlike other fluorescence analyses directed at the detection of the X-rays of several elements simultaneously, the proper choice of materials for the source structure will insure that their fluorescent X-rays do not contribute to the lead X-ray energy region, and the restriction concerning fluorescence of structural materials is minor. The criteria concerning scattering from the source structure, and sample fluorescence to scatter ratio are thus the primary considerations.

One of the significant contributions to background counts in all energy dispersive fluorescence analyses is due to scatter, both coherent and incoherent, of the primary X-rays off structural materials and the sample itself. It is desirable, therefore, to reduce scattering from structural materials to a minimum. Scattering from the sample is of course unavoidable and care must be taken to minimize its contribution to background. The problem of scatter in

relation to the source-sample-detector geometry is another undesirable feature of the arrangements of Figure 3, particularly as it relates to the present problem and the source energies selected. Incoherent or Compton scattering results in a secondary or scattered photon of less energy than the primary photon according to the relationship,

$$E' = \frac{E}{1 + \alpha(1 - \cos \theta)}$$

where E' = energy of scattered photon;

E = energy of primary photon;

$\alpha = E/m_0 c^2 = E/511 \text{ keV}$; and

θ = angle of scatter with respect to direction of primary photon.

In terms of a single scattering event, therefore, the lowest energy secondary photon results from a 180° backscatter. Thus, even though there is only a relatively small energy change when considering the 180° backscatter of the 11.6 keV excitation energy, it would be desirable in terms of the amount of background interference to the lead X rays -- especially the 10.5 keV L_α energy -- to design the source-sample geometry such that the majority of excitation photons scatter at angles of around 90° . This would not only increase the energy increment between the lead X rays and the scatter interference, but also reduce the number of scattered photons. As seen in Figure 4, (10) there is a reduction of a factor of approximately two in the number of photons scattered at 90° as opposed to 180° with an $\alpha = 11.6/510 = 0.0228$.

Variation of the annular configuration to achieve primarily 90° scatter increases the problem of structural scatter and worsens the source-sample geometry, thereby reducing the signal-to-background ratio. Since the annular configuration produces primarily 180° backscatter and does not allow increase of sample-detector geometric efficiency, an entirely different source-sample detector arrangement was designed.

Toroidal Geometry

Previously, we discussed the usefulness of radioisotope sources of excitation because of the number of different configurations it is possible to mold them into. For the present problem, the same is true of the sample in the sense that blood, being a liquid, may be "molded" into any form required. To close the distance between the source and sample, and between the sample and detector, the sample may be formed into a toroidal shape and placed around the source, designed as a central core. The resulting configuration* is shown in Figures 5, 6 and 7. The availability of Pu-238 in the form of PuO₂ microspheres of various diameters from 50 μ to 400 μ(11) made this a practical approach as well as a theoretically desirable one. The sphere size selected for the source is 250 μ, the activity per sphere being calculated as follows:

*Patent info.

$$\begin{aligned}
 \text{Volume} &= 4/3 \pi r^3 = 4.2 (1.25 \times 10^{-2} \text{ cm})^3 \\
 &= 8.15 \times 10^{-6} \text{ cm}^3; \\
 \text{Mass of Pu-238} &= 8.15 \times 10^{-6} \text{ cm}^3 \times 7.35 \text{ gm/cm}^3 * \\
 &= 6.0 \times 10^{-5} \text{ gm Pu}; \\
 \text{Pu-238 activity} &= 6.0 \times 10^{-5} \text{ gm} \times 17.5 \text{ Ci/gm} \\
 &= 1.05 \times 10^{-3} \text{ Ci} \\
 &\text{or } \approx 1.0 \text{ mCi/250 } \mu \text{ sphere PuO}_2.
 \end{aligned}$$

Thus, arranging the microspheres as shown in Figure 7, it is possible to obtain 25 mCi of activity in a 2 mm diameter circle.

As seen in Figure 5, showing the source and sample position with respect to the detector, this configuration allows irradiation of the sample throughout the 360° in the plane of the source, brings the source to within a distance of approximately 1 mm from the surface of the sample and the sample to about 1 mm from the entrance window of the detector cryostat. The detector position within the cryostat is fixed by the manufacturer at a minimum distance of 5 mm from the entrance window. The approximately 1 mm collimation space between the source and sample is necessary to prevent direct transmission of the Pu X-rays to the detector. Also,

*Density of Pu-238 in product as given by Mound Laboratories.

by placing the source inside the sample, external structural material is eliminated thus limiting scattering interference to the sample itself and a minimal amount of scattering and fluorescence from the edges of the source holder. Since the holder is constructed of nickel, however, its fluorescence X-rays are not expected to interfere in the 10.5 keV Pb region. Also, because of the direction of Pu X-rays with respect to the detector, the scattering will be predominantly 90°, thus taking advantage of the reduction in the number of photons scattered at that angle in the energy region of interest, and the increased energy separation between 90° scatter and the Pb fluorescence energies, as discussed.

This configuration thus minimizes background due to scattering and fluorescence of external structural materials while increasing the signal through the closest possible proximity of both source-sample and sample-detector.

Estimates of geometrical efficiency for this configuration are approximately 11% for source to sample and 10% for sample to detector, with an 8 mm diameter entrance window on the cryostat as shown.

Due to previous commitments of personnel at Mound Laboratories, there was a delay in the construction of this source and an alternate design was constructed by Amersham-Searle (shown in Figure 8). This configuration utilized five 1 mm diameter beads, each containing 4 mCi Pu-238 for a total activity of 20 mCi sandwiched between the 0.5 mm Cu

discs. To keep the sample size uniform, this source was also designed to be 4 mm in diameter and because of the 1 mm spacing of the Cu discs, the collimation is not as right as in the first design. To avoid direct transmission of the primary X rays, therefore, this source was raised off the detector window surface to a height of approximately 4 mm in diameter and because of the 1 mm spacing of the Cu discs, the collimation is not as tight as in the first design. To avoid direct transmission of the primary X rays, therefore, this source was raised off the detector window surface to a height of approximately 4 mm, decreasing sample-to-detector geometry to about 4%. There is also more self-absorption in the larger 1 mm diameter beads than in the 250 μ beads because of the greater mass of plutonium present.

In summary, the optimum design choices are:

- i. a lithium-drifted silicon detector (Si(Li));
- ii. a Pu-238 source; and
- iii. geometry as indicated in Figure 5.

B. Results:

1. Definition of Major Parameters: The 20 mCi Pu-238 source, fabricated for us by Amersham-Searle, was used to obtain some preliminary results and demonstrate the feasibility of this geometry for the excitation and detection of sub- $\mu\text{g/ml}$ levels of lead in blood.

a) Sample Preparation

The blood samples used for the calibration and sensitivity experiments were made from "normal", human blood with a known amount of standard lead solution added. The blood was drawn, by capillary action, into a 75 μl hematocrit tube and then placed, drop by drop, into an absorbent material pre-cut in the form of a toroid. The material used for the toroidal-shaped blood retainers were millipore absorbent, or nutrient, pads, made from pure, white cellulose material which resembles blotting paper and is approximately 0.8 mm thick. No extraneous interfering fluorescence peaks have been noted from this material.

The toroids are cut with an 8 mm outer diameter and a 4.2 mm inner diameter which allows the sample to slip easily over the 4 mm diameter source. The crosshairs which hold the source in position also keep the sample in position around the beryllium window of the source.

During preparation, the toroid sample is held on a 4.2 mm diameter shaft with base fixture, cut from a teflon rod to which the blood does not adhere. The filter after addition of the sample, is placed under a heat lamp to dry while still

on the teflon shaft. Adequate drying is accomplished in 3 to 4 minutes, after which the sample is removed, ready for fluorescence analysis.

b) Detector Calibration

The energy calibration curve for the Si(Li) detector used for the experiments is shown in Figure 10. The calibration source used was Co-57 with photon energies of 6.399 keV, 7.057 keV and 14.40 keV. The offset at channel zero is due to the fact that the multichannel analyzer used has no zero level adjustment. With this calibration, the midpoint of the 10.5 keV Pb L X-ray peak appears in channel 270.

A spectrum of the source material is shown in Figure 11. This was done by placing the source with the beryllium window facing the detector at a height of approximately 15 cm above the detector. Because of the gain adjustments chosen in the calibration, the U-L_α's,* with energies in the 20 keV range, are off scale and not shown in the figure. Clearly seen, however, are the U-L_{β₁} (17.2 keV), U-L_{β₂} (16.4 keV), U-L_α's (13.618 and 13.446 keV) which appear as a single peak at 13.6 keV, and the 11.6 keV U-L₁ peak. The less abundant U-L_η and U-L_{β₆} peaks are also shown. The peaks appearing below the uranium X-ray peaks are the Cu K_α and K_β peaks (8.04 and 8.95 keV) from the source holder-collimator and smaller K_α peaks of Fe and Ni (6.4 keV

*The X rays observed are emitted, for the most part, from the uranium daughter product of the alpha decay of plutonium. Hence, the terminology, U-L_α, etc.

and 7.47 keV) due to fluorescence of the detector cryostat. The Pb K_{α} peak integration region is indicated in the flat portion of the spectrum below the U- L_1 peak.

Of interest are the height of the uranium X-ray peaks relative to each other. Theoretical calculations of the relative intensities of the various uranium L X-rays (12) show the U- L_{β_2} intensity to that of the U- L_{α} lines to be approximately 136/111, considering the L_{α_1} line to be 100 and the L_{α_2} line to be 11; also, the U- L_1 intensity relative to the L_{α} lines is approximately 7/111. However, as seen from Figure 11, the observed ratio of the peak heights of the U- L_{β_2} /U- L_{α} and U- L_1 /U- L_{α} are 4750/2160 and 90/2160 respectively, indicating the rather strong self-absorption factor of the source.

The effects of scatter, both coherent and incoherent, may be seen by comparing Figure 11 with the several spectra shown in Figure 12. The spectra in Figure 12 were taken after the addition of approximately 1 mm of gold metal to the face of the source towards the detector. The addition of the gold was necessitated by the unexpectedly large contribution to background from the 60 keV gamma-ray of Am-241. This nuclide, which grows into every plutonium source from the decay of Pu-241, is unavoidable, but has much less of an effect in a source of freshly separated material when ingrowth time is short. It is believed that

the Pu material used for this source is 6 to 8 years old allowing almost maximum ingrowth of the Am-241. The effect of the 60 keV scatter is shown in Figure 13, which is a spectrum of the source in position without the gold in place. The increase in background levels beyond channel 300 (11.6 keV) is believed due to 60 keV photons Compton scattered from the cryostat materials surrounding the detector. The rather rapid increase at and below channel 300 is due to Compton scatter in the detector itself in which the maximum energy deposited by a Compton electron in a 180° backscatter event is calculated to be 11.5 keV. This effect, in addition to the fact that the relatively wide (1 mm) spacing of the collimator lips allowed the primary photons to interact with the detector when the source was near the surface of the detector entrance window, necessitated raising the source away from the desired position as shown previously in Figure 5. The optimum height above the surface of the entrance window, as determined by measurements of $(\text{signal})^2/\text{background}$, for the source with the additional gold absorber is 3.5 mm, as shown in Table III. These data were obtained using a blood sample containing 1.0 μg of lead/ml of blood. The height measured is the height of the crosshairs, corresponding to the bottom surface of the toroidal blood sample. The spectra shown in Figure 12 were obtained with the source at this height.

Table III

<u>Height above Surface</u>	<u>S²B</u>
1.5 mm	31.5
2.0 mm	37.95
3.0 mm	41.2
3.5 mm	42.3
4.0 mm	31.8
4.5 mm	12.9

As seen from spectrum (a) of Figure 12, a spectrum of the source in position without any absorbing or scattering material around it, the background level in the 10.5 keV Pb L_α region has been reduced by a factor of approximately four with the addition of the gold to the bottom of the source.

Unfortunately, the gold also contributes to the background with X-rays at 9.7 keV (L_α) and 11.5 keV (L_β). The Au L_α contribution is seen as a shelf on the upper side of the Cu K_β peak, while the 11.5 keV Au L_β line is masked by the scatter peak of the 11.6 keV U-L_α X-ray. The effects of moving the source closer to the detector are seen in the large increase in the height of the Cu peak from the source structure and the height of the Fe K_α and K_β peaks and the appearance of the K_α peak of chromium due to the cryostat material. As seen, the well-defined uranium X-ray peaks have disappeared and been replaced by the broader peaks of their scatter counterparts. The effect on the height of these scatter peaks due to an increase in low Z scattering material is seen in spectrums (b), (c), (d) and (e) of Figure 12.

Spectrum (b) is the source in position as in (a), but with a blank toroid surrounding it. The scatter peaks are now clearly defined with increases of approximately a factor of 4, 3 and 2 in the heights of the $U-L_{\beta}$, L_{α} , and L_{γ} respectively compared to the source alone. The positions of the various scatter peaks also corroborate the prediction of predominantly 90° scatter for which the source geometry was designed. A further increase in the amount of scatter is seen in the scatter peak heights of spectras (c), (d), and (e) which are spectra of 75 μ l of the blood of a baby baboon, 75 μ l of "normal" human blood and 75 μ l of human blood with 10 ppm lead added. The blood of baboons has been found to be quite similar chemically to human blood (13). In addition to this similarity, the blood of newly imported baby baboons contains < 0.05 ppm Pb (13). We were able to obtain a small amount of this blood recently from a baby baboon imported approximately one month before the sample was received and have used it to establish a "lead-free", blood background count-rate.

Immediately apparent in the blood spectra is the large increase in height of the fluorescent Fe K_{α} and K_{β} peaks from the Fe normally present in blood at a level of approximately 50 mg/100 ml. These peaks are probably somewhat enhanced by the Cu X rays from the source which are near the absorption edge of Fe and therefore also induce fluorescence from the Fe in the sample, as well as in the structural material of the cryostat. The other peaks present in the blood spectra are potassium and chlorine, both normally present in

blood at levels of approximately 200 mg/100 ml respectively, and the 11.9 keV K_{α} peak due to bromine, present in human blood at levels of approximately 0.9 mg/100 ml.

The Pb L_{α} peak at 10.5 keV is discernible in the spectrum of normal blood, above the background as seen in the spectrum of the baby baboon blood. The Pb K_{α} peak at a level of 10 ppm is, of course, obvious with some evidence also of the 12.6 keV Pb L_{β} peak. It should be noted that the spectra of Figure 12 are the average of ten, one-minute counts, hence the statistics are somewhat improved and account for the visibility of the Pb K_{α} in the "normal" blood sample. This is not true for a single, one-minute count, which was the original goal of the work. The "normal" level seen in spectrum (d) has been estimated to be 0.43 $\mu\text{g/ml}$ or 43 $\mu\text{g/dl}$.

c) Sensitivity and Detection Limit

The system sensitivity and detection limit for the measurement of lead in blood using the 20 mCi, Amersham-Searle source were determined by adding known amounts of a standardized lead solution to measured amounts of "normal" blood. The standard lead solution contains 1 mg Pb/ml. One tenth ml of standard, i.e., 100 μg Pb, was added to 10 ml of blood to make a solution containing $100 \mu\text{g}/10.1 \text{ ml} = 9.9 \mu\text{g/ml}$. Nine blood solutions were similarly made containing 0.5, 1.0, 2.0, 3.3, 4.3, 5.0, 6.2, 7.4, and 9.9 $\mu\text{g/ml}$ of Pb.

Blood samples for count rate determinations were made in toroid form using aliquots of 75 μl aliquot of "normal" blood with no added lead, also counted 10 times, was then subtracted from the nine samples. The results of these determinations are shown in graph form in Figure 14. The resultant curve was computed as a straight line ($\rho = 0.994$), with a slope of 29.7 cpm/ $\mu\text{g}/\text{ml}$.

The value of background used below to calculate the detection limit of the system was obtained from the spectrum of the baby baboon blood. The method used is that of Pasternack and Altshuler (11) and has the form:

$$\text{MSA} = \frac{K_{\alpha}}{s} \cdot \sqrt{B/t}$$

where: MSA = minimum significant measured amount;
 K_{α} = constant determined by level of significance desired and obtained from statistical tables;
 s = sensitivity in cpm/ppm $\approx$ 30 cpm/ppm;
 B = background = 315 cpm; and
 t = total counting time of sample.

The detection limits (MSA's) calculated and shown in Table IV are based on a level of significance of 0.05

TABLE IV
Detection Limits for 20 mCi
Amersham-Searle Source

Time (min)	Detection Limit		
	ppm	$\mu\text{g/ml}$	$\mu\text{g/dl}$
1	1	1	100
2	0.7	0.7	70
5	0.4	0.4	40
10	0.3	0.3	30
20	0.22	0.22	22

The source used for the preliminary work was a compromise which did not fulfill all the initial design criteria. Use of the larger 1 mm diameter spheres increased the collimator spacing, causing scattering conditions to change and increased self-absorption of the primary X rays. The necessity of adding the gold to the bottom of the source to absorb the excess amount of Am-241 50 keV photons, along with the required increase in spacing between the sample and the detector, had the effect of decreasing the estimated sample-detector geometry. Nevertheless, the 20 mCi Amersham-Searle source did provide experimental evidence of the efficiency of the basic source holder-collimator design, indicating the possibility of detecting sub-ppm levels of lead in blood with a minimum amount of sample preparation, no chemical manipulation, and a reasonably short counting time. While the goal of a 0.2 $\mu\text{g}/\text{ml}$ detection limit for a 2 minute count was not attained with this source, the limit of 0.4 $\mu\text{g}/\text{ml}$ with a 5 minute count represents about a 35 fold decrease in required counting time when compared to a 1971 publication (14) which indicated a detection limit of 0.5 $\mu\text{g}/\text{ml}$ with 180 min. counting time, also using radioisotope excitation for X-ray analysis of lead in blood. Since no indication was given as to the geometry, type of source, source strength, or sensitivity, a direct comparison of systems could not be made.

2. Method Development

The results presented above indicated that, although the toroidal geometry offered a considerable increase in geometrical efficiency over other source-sample-detector configurations, a large increase in signal/background ratio was still required if the detection limit of 20 $\mu\text{g Pb/dl}$ was to be attained with analysis times of the order of a few minutes. To increase the signal without unduly increasing the sample size required, effort was focused on improved sample preparation techniques and optimization of source design. This effort resulted in several changes, both in sample preparation and source design, which are described briefly below.

a) Sample Preparation

As discussed above, the first preparation technique consisted of placing 150 μl of whole blood onto a form produced from Millipore filter backing material. The form was produced by cutting the absorbent backing materials in the shape of a flat, concentric cylinder using a special, circular razor made by clamping two pieces of a razor blade cut to the proper lengths and placed into a slotted jig to provide two sharp cutting edges in the forms of circles, one of about 4.2 mm and the other of about 8 mm in diameter. The blood sample

was then placed on the form (dropwise) and dried under a heat lamp. During drying the form was held on a non-wettable, teflon shaft through its center hole to keep the inner diameter of the sample constant.

Although sample preparation was quick and simple with this technique, several problems were noted which affected the reproducibility of the sample and hence the X-ray production rate from a given lead concentration.

These were: 1) thickness of the sample varied from sample to sample causing a change in the geometric cross-section as viewed from the source, thus causing the scattered background to vary from sample to sample. 2) As the sample dried under the heat lamp, the center hole tended to shrink and grip the teflon post tightly making the sample difficult to remove and causing occasional loss of the dried, brittle sample through breakage. 3) The material on which the sample was being absorbed, although it did not produce any interfering elemental peaks, did serve as a scattering mass in addition to the blood sample and served to increase background by its presence.

An alternate preparation technique was sought, therefore, which would allow the making of samples of reproducible geometric dimensions and, in addition, eliminate the need for a form on which to place the sample

and, thus, eliminate that source of background, i.e., additional scattering mass. This, it seemed, could be achieved by drying the blood sample in a teflon container to which it would not adhere. The teflon container developed consisted of a hollow cylinder of inside diameter approximately 9 mm, which fit tightly over a teflon base piece from which a central post of 4 mm diameter protruded and around which the sample would dry, within the cylinder. After drying, the sample was pressed into the final cylindrical shape by a teflon plug which was machined to fit over the central post and down tightly into the cylinder, deep enough to produce a sample thickness of approximately 1 mm to 1.5 mm thick. To produce a dried sample of this size, the original sample volume was increased from 150 μ l to 400 μ l, an amount more difficult to get but still obtainable with an energetic finger stick technique.

The preparation procedure using the teflon "mold" consisted of placing the blood sample into the hollow formed by the joined cylinder/central post teflon pieces and placing under the heat lamp to dry. When the sample was "nearly" dry, i.e., having a clay-like consistency, the plunger portion of the teflon mold was pressed into place to form the desired shape. The sample was then removed by withdrawing the central post, leaving the

sample in place in the cylinder, and then pressing the cylinder fully onto the plunger thereby pushing out the sample. The formed sample was then placed under the heat lamp to fully dry.

Calibration runs of "spiked" blood samples produced by this technique resulted in a sensitivity of approximately 60 cpm/ppm/ $\mu\text{g}/\text{ml}$ and a background of 350 cpm in the Pb-L_{α} X-ray region of the spectrum. This is compared to the initial preparation technique wherein the 150 μl sample yielded 30 cpm/ $\mu\text{g}/\text{ml}$ and a scatter background of 315 cpm. The increase in sensitivity was expected with the increased sample size. And, although the sample size did increase by greater than a factor of two, the background increased only slightly (315 to 350 cpm) because the scattering mass increased only slightly - the original absorbent material being replaced by the self-supporting sample matrix itself.

Comparing the detection limits of the two preparation techniques, the teflon molded, 400 μl sample yields, at the 0.05 significance level, a detection limit of 20 $\mu\text{g}/\text{dl}$ with a counting time of approximately 6 minutes. Recalling the results shown in Table IV for the previous preparation technique - 22 $\mu\text{g}/\text{dl}$ in 20 min. - this was an improvement of a factor of three.

Although the use of the teflon mold produced samples which were more consistent than the original technique and resulted in an improved detection limit, further modifications in sample preparation were necessary because some of the samples were being partially discharged up the plunger/cylinder interface when pressed into shape. This was due, in part, to the difficulty of obtaining a consistent time to the "nearly" dry point when the teflon plunger would be inserted, and, in part, to the great amount of pressure being applied by the hand-held, KBr pellet-press into which the teflon jig was inserted to press the sample into its cylindrical shape. To circumvent this problem, the teflon was replaced by a jig machined out of aluminum. The sample, now dried separately on a small piece of teflon under the heat lamp was introduced into the holder via a teflon funnel, machined to fit tightly over the central hole of the aluminum jig, to prevent the loss of any of the now brittle sample as it is pressed into the jig opening.

Because the sample was now much drier, more pressure had to be exerted by the KBr press, eventually causing the aluminum jig to distort and become unopenable. In addition, it was found that the sample was often still somewhat moist in the center, due to the fact that the infrared lamp would dry the surface first and occasionally leave the center of the sample mass undried again resulting in sample loss up the sides of the aluminum.

Subsequently, a more thorough and, coincidentally, more rapid drying was obtained by using a microwave oven. Since these devices emit electromagnetic radiation at a frequency that is preferentially absorbed by water, the blood sample was now quickly and thoroughly dried, eliminating the occasional loss of sample due to uneven drying under the heat lamp. The problem of distortion of the jig during pressing of the dried sample was eliminated by replacing the aluminum jig with one machined out of steel.

b) Source Design

During the time of the initial calibration and measurement tests of the 20 mCi Amersham source and the subsequent refinement of the sample preparation methodology, the 25 mCi source utilizing the microspheres from Mound Laboratories was completed. Initial tests on this source showed some design defects similar to the 20 mCi Amersham source. As seen in Figures 5 and 6, the top and bottom support lids have the same diameter, viz., 4 mm. When the source was placed in position as indicated in Figure 5, scatter of the source X rays off the edge of the upper support lid directly into the detector was copious. To eliminate this, it would have been necessary to raise the source position above the detector as was necessary with the Amersham source which would have resulted in little or no net gain in sensitivity with the Mound source.

To overcome the scatter problem engendered by the upper support lid, a refined design was produced based on the experience gained with the 20 mCi Amersham and 25 mCi Mound sources. As seen in Figure 15, the new source design allowed for a recessed upper support lid and resulted in a significant reduction in the amount of scatter from that surface directly into the detector. Also to be noted is the slightly increased spacing between the upper and lower support lids. This change was made to accommodate a second row of 25 microspheres, or a total of 50 microspheres of 1 mCi each to produce a 50 mCi source with approximately the same dimensions as the original 25 mCi design. The microspheres were subsequently replaced with PuO_2 "shards" (during the manufacture of the source.) These are small, uneven pieces of the same material used in the microspheres and would insure against the possible motion of the microspheres within the source after sealing. The activity of the new source was estimated to be approximately 58 mCi by Mound personnel who constructed it. Unfortunately, this amount of activity caused significant amounts of scatter off the sample itself and necessitated the use of the Au cap as shown in Figure 16A to reduce the sample/detector solid angle and dead time losses in the amplifier-analyzer system.

To take advantage of the increased collimator spacing between the source and sample incorporated in the new source design, a further refinement was made to the sample shape. As seen in the drawing (Figure 16A), the cross section was changed from rectangular to trapezoidal shape.

The results of sensitivity tests with the new 50 mCi Mound source and the modified toroidal sample shape are shown in Table V compared with the previous results obtained with the Amersham 20 mCi source. Although the sensitivity did not increase as much as expected with the new 50 mCi Mound source due to the Au cap, it did increase from 60 to 100 cpm/ $\mu\text{g}/\text{ml}$ with only a relatively small increase in background, from 350 to 400 cpm.

Thus, with the refined source design and the trapezoidal sample shape, the system is capable of detecting 0.2 $\mu\text{g}/\text{ml}$ with a 2.5 minute counting time, an improvement of a factor of 10 over the original system.

3. System Calibration

a) Whole Blood Samples

At each stage of the development and refinement of the sample preparation technique and with source design changes discussed above, intercomparison experiments

TABLE V

<u>Source</u>	<u>Sample Form</u>	<u>Bkgd. (cpm)</u>	<u>Sens. (cpm/μg/ml)</u>	<u>Time to Detect* 0.2 μg/ml</u>
20 mCi Amersham	150 μ l, dried, on filter form	315	30	25 min.
20 mCi Amersham	400 μ l, dried, no form	350	60	6 min.
50 mCi Mound Lab.	400 μ l, dried,	400	100	2.5 min.

*Calculated at 1.64 σ , i.e., 0.05 significance.

were carried out in which measurements were made of the concentration of lead in non-human primate blood by A.A. spectroscopy and the XRF method. The primates (baboons) were part of another experiment being conducted at the Institute of Environmental Medicine in which these animals ingested or were injected with varying amounts of lead-based compounds (15).

Although these intercomparisons showed some results in good agreement, large differences were still seen in the results of sample measurements with the two methods, even though the calibration of both systems with known amounts of lead added to "normal" blood yielded curves which were reproducibly linear.

The clue to the differences in results obtained with the two systems came with the observation that the weight of the dried blood sample was linearly correlated with the hemoglobin content as well as the hematocrit of the sample. Both of these indices are, of course, directly related to the red cell mass of the sample where the majority of the lead is contained. Subsequently, it was observed that the difference in net counts between the actual XRF sample measurements and a calculated count obtained from an independent measurement (A.A.) of the lead in the sample multiplied by the XRF sensitivity factor (cpm/ μ g/ml) was also linearly related to the weight of the

dried blood sample. These observations were thought to be of basic significance in explaining the differences in results between the two methodologies and, therefore, a new and more detailed calibration procedure was carried out as described below.

i) Calibration Procedure and Results

Calibration standards were prepared by adding known amounts of lead in solution to six aliquots of infant baboon blood, each previously diluted or concentrated to different hematocrit values and hence, hemoglobin contents. The hematocrit values selected were 24, 30, 36, 42, 48 and 56%. Each hematocrit level was spiked with different amounts of lead to obtain 0, 0.2, 0.4, 0.7 and 1.0 $\mu\text{g/ml}$ added. From each of these aliquots, a portion was taken for A.A. analysis and three 400 μl samples were dried and pressed into toroids for XRF counting.

The weight of the dried, pressed blood toroid is shown for varying levels of hematocrit and hemoglobin, respectively, in Figure 17. The weight is a linear function of hematocrit and/or hemoglobin content. The variation in weight over the range of hematocrits chosen is approximately a factor of 2.

The count rate versus $\mu\text{g}/\text{ml}$ of lead contained in the samples for one of the hematocrit aliquots (30%) is plotted in Figure 18. The abscissa values of $\mu\text{g}/\text{ml}$ contained in each sample were obtained from A.A. spectrometer measurements as mentioned earlier. This curve, and the other five similar curves for the rest of the hematocrit values, were used to extract two pieces of information: the slope, i.e., $\text{cpm}/\mu\text{g}/\text{ml}$ and, by extrapolation, the background count rate of a sample of blood containing no lead, as a function of hematocrit value and hence as a function of the weight of the sample.

A plot of both the zero background, i.e., the expected count rate of a sample containing no lead; and the sensitivity factor or slope in terms of $\text{cpm}/\mu\text{g}/\text{ml}$ is shown, versus weight, in Figure 19. The correlation coefficients and the equations of the fitted curves (least squares regression) are shown on the plot. The sensitivity factor decreases by approximately 20% over the sample weight range and the zero background increased by approximately 40%. Because previous calibrations were performed using normal blood samples whose hematocrits always fell within a reasonably narrow range, the above

effects were not observable and large errors were thus possible if the unknown sample being measured had a hematocrit which fell outside the range of the calibration standards used. For the sensitivity factor curve, $y = ae^{-bx}$, the factor $(-b)$ is small enough (0.0052) that the curve could be approximated by a straight line. In fact, a straight line, least squares regression yields a fit of 0.75 for the same points and a power curve fit is only slightly better at 0.79. The exponential is used intuitively because the process being observed is predominantly one of attenuation of the 10.5 keV Pb K_{α} X ray.

Calculations have performed in an attempt to account for the changes observed in background and sensitivity using the known constituents of blood, and the equation

$$\mu/\rho = \frac{\mu_1}{\rho_1} \omega_1 + \frac{\mu_2}{\rho_2} \omega_2 + \dots + \frac{\mu_n}{\rho_n} \omega_n$$

where: μ/ρ is the overall mass attenuation coefficient of the blood;
 μ_1/ρ_1 etc. are the mass attenuation coefficients of the individual elements; and
 ω_1 etc. are the fractions by weight of the elements that make up the blood.

Applying this equation to trace elements with concentrations in blood greater than 0.1 mg/100 ml and hemoglobin ($\text{Hb} = [\text{C}_{738}\text{H}_{1166}\text{FE N}_{203}\text{O}_{208}\text{S}_2]_4$), calculations were made to obtain incoherent and coherent scattering coefficients at 11.6 keV as well as the photoelectric absorption coefficients at 10.5 keV (Pb K_α) for hemoglobin values of 7.6, 11.5, and 15.7 gm/100 ml. These were the measured values corresponding to hematocrits of 24, 36, and 48% and weights of 59.5, 70.6 and 83.1 mg, respectively. The 11.6 keV U_ℓ source energy was chosen because, due to resolution, it has the most effect on the 10.5 keV Pb background.

The absorption coefficients, using the equation $N/\text{No} = e^{-Tx}$, where T = absorption coefficient, x = sample thickness, predict a change in sensitivity factor of approximately 8%, whereas the change seen in Figure 19 is closer to 20%. This difference is thought to be due to poor experimental technique in the first calibration experiment. (Hct = 24, 36, 48) in which it is believed that inefficient mixing of the blood between pipetting may have occurred, resulting in too great a change in slope with change in hemoglobin. Fortunately, the change of sensitivity factor over the entire weight range is not as critical

as the change in zero (Pb-free) background which appears not to have been affected by poor mixing, because the blank blood (i.e., 0 ppm added) was always sampled first to avoid contamination. Calculations done as indicated earlier show a change in scattering coefficient at 11.6 keV of about 34% over a weight range of 59.5 mg to 83.1, and the actual change in zero background is approximately 30% over the same range. The second experiment (Hct = 30, 42.5, 56), in which a gentle vortexing was done between sampling, resulted in a more gradual slope change with increasing hematocrit, and accounts for the relatively poor correlation of the fitted exponential to the points shown in Figure 19.

The weights of the dried samples, hemoglobin content and hematocrits were obtained using standard laboratory apparatus. For field measurements, however, this additional equipment would not be necessary. For the calibration, two single channel analyzers were used, one set to record counts from the lead region of the X-ray spectrum and the other set up to monitor the iron region. As seen in Figure 20, the counts in the Fe channel are proportional to the weight of the sample. This is as expected, since the Fe is

contained in the hemoglobin, which makes up approximately 95% of the dry weight of the red-cell and is therefore proportional to the dry weight of the sample. Thus, the weight of the sample may be estimated from the counts in the Fe channel. Moreover, with proper calibration, the Fe content in milligrams may be obtained simultaneously with the Pb.

ii) Intercomparison of XRF and A.A.

Measurements

The results of intercomparison measurements between the XRF system using the above calibration results, and A.A. (Delves cup) spectroscopy in our laboratory are listed in Table VI. The blood samples, several from each, were obtained from infant baboons who had ingested lead in various forms and in various amounts over a period of time as part of a lead toxicity study (15).

Shown are the date the sample(s) was taken, the animal identification number, and the A.A. and XRF results. Each such sample was counted twice for 10 min. The number in parentheses beside each XRF result represents the number of individual 10 min. counts, and therefore twice the number of samples from each animal. The amount of blood available from each animal for the XRF analysis

TABLE VI

Interlaboratory Comparison: Baboon Blood

Sample Number	Atomic Absorption (ppm)	X-ray Fluorescence Analysis (ppm)
804	0.24	0.06±0.09 (4)
243	1.2	1.41±0.08 (4)
227	0.11	0.02±0.06 (2)
255	0.53	0.68±0.07 (4)
788	0.65	0.77±0.11 (4)
257	0.34	0.28±0.08 (4)
225	0.36	0.44±0.07 (4)
249	0.37	0.47±0.12 (4)
724	0.11	0.20±0.08 (4)
802	0.19	0.16±0.12 (4)
796	0.71	0.86±0.11 (4)
243	1.95	1.75±0.09 (2)
257	0.35	0.46±0.08 (4)
227	0.12	0.17±0.09 (4)
796	0.79	0.74±0.10 (6)
227	0.13	0.19±0.04 (6)
786	0.71	0.95±0.11 (6)
802	0.13	0.18±0.07 (6)
243	2.88	2.73±0.16 (6)
257	0.52	0.47±0.08 (6)
786	0.78	0.89±0.05 (6)
796	0.74	0.78±0.08 (6)
257	0.44	0.50±0.12 (2)
227	0.11	0.11±0.07 (6)
243	2.96	3.07±0.22 (8)

varied, depending on the amount of blood needed for the other tests being performed; therefore, the number of samples varied. (The errors shown are one standard deviation.) As seen, the XRF measurements now compare quite well with the A.A. results. The weight for these samples varied over the entire range shown in Figure 19. Since for each end of the weight range the counting error due to background alone is equivalent to 0.06 ppm and 0.09 ppm, respectively, the errors shown indicate that other errors (position of sample, sample preparation, etc.) are smaller than the counting error.

With the cooperation of Dr. Bernard Davidow, some intercomparison measurements were performed with the New York City Department of Health, whose chemists do hundreds of analyses per day of lead in children's blood using an automated A.A. spectroscopy system.

The blood lead values for the above sonicated samples obtained by XRF versus the NYC Health Department A.A. results are shown in Table VII. These results are in good agreement with the A.A. results.

TABLE VII

Intercomparison Test: Children's Blood

<u>Sample No.</u>	<u>XRF (w/o Weight Correction)</u>	<u>XRF (with Weight Correction)</u>	<u>NYCDH (A.A.)</u>
25	0.01±0.05	0.16±0.05	0.20
27	0.05±0.09	0.18±0.08	0.17
28	0.40±0.06	0.52±0.05	0.68
29	0.12±0.09	0.25±0.08	0.19
30	0.02±0.06	0.16±0.06	0.17
56	0.51±0.13	0.64±0.13	0.60
62	0.21±0.06	0.35±0.06	0.40
69	0.13±0.11	0.32±0.12	0.30
82	0.12±0.10	0.27±0.10	0.23
86	0.08±0.14	0.19±0.18	0.20
88	0.46±0.16	0.57±0.17	0.64
103	0.42±0.09	0.56±0.09	0.52

b) Separation of Red Cells and Plasma

These results, showing the relationship between changes in hematocrit and/or hemoglobin and the background and sensitivity factor for the measurement of Pb in blood, are of basic significance to the quantitative assessment not only of Pb but of trace metals in general in body fluids and other tissue by X-ray fluorescence analysis. Differences in blood parameters like hemoglobin and hematocrit will be present because of natural biological variability or, more importantly, the body's response to various disease mechanisms. It is important to account for such changes by calibration, as above, or, if possible, eliminate them in order to maximize measurement precision.

With this in mind, effort was directed toward the development of a field technique for the separation of the blood sample into two fractions, red cells and plasma, with each fraction being prepared in turn as a dried, torroidal specimen. Since the weight of the blood sample is proportional to hematocrit, and thus to the number of red cells, the changes in scattered background and sensitivity observed using whole blood samples are believed to be due to a varying specimen density -- the result of pressing a varying sample weight into a fixed volume. Separation of the blood into two fractions -- red blood cells (rbc's) and plasma -- would allow the preparation

of specimens of constant density (regardless of hematocrit) by drawing off fixed volumes of rbc's and plasma from the separated fractions. In this manner, the changes in background and sensitivity resulting from variations in hematocrit, i.e., packed red cell volume, are eliminated. Also, the separation technique would concentrate those trace elements which may be predominantly present in one or the other fraction, such as Ca in plasma, and K, Pb and Fe in the red cell, and facilitate measurement of the intra-to extra-cellular ratio of those elements such as Na, Cl, K and Ca, whose transport from one fraction to the other may be effected by certain disease states. In addition to the separation technique for constant weight samples, a new specimen geometry was designed to increase the output of low energy X rays of the elements Ca, K, etc.

Depending on the individual's state of health at any given time, hematocrit values can easily range from 0.25 and less in anemia to 0.50 and greater in polycythemia. Therefore, to maintain a fixed volume of rbc's upon separation, it is necessary to fix on that volume at the lowest hematocrit expected. Since the best sensitivity obtained thus far had been with a 400 μ l sample volume, which at a normal* hematocrit of around 0.36

*Children between the susceptible ages of 1 to 6 years have "normal" hematocrits of about 0.36; adults' "normal" is about 0.43 (8).

would contain 144 μl of rbc's, this volume of rbc's would be necessary to maintain that sensitivity. To do this, the whole blood sample volume would have to increase to 720 μl to yield an rbc sample of 144 μl at a hematocrit of 0.2. For practical purposes, 700 μl was used because of the availability of Wintrobe tubes of that volume conveniently marked off in millimeter steps from 0 to 100. Several attempts to use the small volume, capillary hematocrit tubes failed because of the difficulty of removing clean samples of rbc's and plasma from these small bore tubes. The Wintrobe tubes, with I.D. = 3 mm, allowed the removal of both fractions using Pasteur pipettes and an easy, reproducible technique was developed for obtaining fixed-volume samples of each fraction (see Appendix A).

Although 140 μl of rbc's may be obtained from 700 μl of whole blood at hematocrit = 0.2, 120 μl was used to avoid the possibility of accidental mixing at the interface. Similarly, a fixed plasma volume of 350 μl was used to allow for a 700 μl sample size with a hematocrit of 0.5.

The effectiveness of the separation procedure to provide samples of constant weight was tested with 20 replicate samples each of 120 μl rbc's and 350 μl plasma, dried and pressed in the new geometric form described below. The specimen weights were 43.0 ± 1.4 mg and

36.0 $\pm$ 1.9 mg for the rbc's and plasma respectively. The difference in water content of the two fractions ($\sim$ 90% in plasma, $\sim$ 64% in rbc's) is reflected in the dry weights. These weight ranges are contrasted with the previous 400 μ l whole blood samples which had a dry weight which ranged from 60 to 100 mg depending on hematocrit.

i. New Specimen Geometry

The geometric configuration of the pressed specimen used until now had been designed to maximize the signal-to-background ratio of the 10.5 keV Pb L _{α} X ray. This geometry is shown in a cross-sectioned view in Figure 16A. To increase the output of the lower energy K _{α} X rays of Cl, K, and Ca from the sample, a new specimen geometry was designed as shown in Figure 16B. In cross section, it has the shape of a truncated, hollow cone. Although it increased the distance between the blood and the source, this form also has a much larger surface area for excitation and emission of the K X rays of the lower Z elements, and, as will be shown, effectively increased the signal to background ratio for these energies.

A further increase in signal/background at these energies was realized with a smaller sample size (400 μ l) and the use of a smaller (80 mm²)

detector with improved resolution. These changes will be discussed in the multi-element calibration section of this report. Before that, however, the results of Pb calibrations with the new geometry will be described and compared to the previous Pb calibration results.

ii. Red Cell Pb Calibration - 700 μl whole blood/120 μl rbc/200 mm^2 detector

As shown earlier, Pb calibrations using whole blood samples were done by adding fixed amounts of standard Pb solution to blood to make up a calibration curve from which the slope, in $\text{cpm}/\mu\text{g}/\text{ml}$ could be determined. In addition, the blood used (infant baboon blood) had been shown to contain very small concentrations of Pb and therefore also served as a Pb-free background standard. The use of separated samples of rbc's and plasma created the need for a new calibration technique and a substitute material that would yield a background count rate in the Pb region equivalent to the Pb-free blood sample used previously. Although the "Pb-free" blood could again have been used as background, it was decided to find a substitute that would service as a matrix to which could be added, in addition to Pb, measured amounts of all of the elements seen in the blood spectrum -- it would be difficult to

make standard additions of the elements to the separated fractions of a blood sample -- as well as a trace element-free matrix for background correction.

Millipore filters of the mixed cellulose ester type seemed to fulfill both requirements above, i.e., the scattered background spectrum appeared to be a good match for the rbc and plasma spectra when the spectrum of pressed filter samples of the same weight were compared to them; and, in addition, Pb, or any element in liquid form, could be simply added to them in measured amounts for calibration. Measured amounts of Pb were added in this manner to a filter paper matrix weighing 43 mg to match the dry weight of the 120 μ l rbc fraction. Using a blank, 43 mg filter paper matrix as background, the Pb calibration factor using the new geometry was 360 cpm/ μ g Pb with a background of 410 cpm. At a significance level of 0.05 (1.64σ), this yields a detection limit, for a 2 min. counting time, of $(1.64\sqrt{410/2})/360 = 0.065 \mu\text{g Pb in } 120 \mu\text{l of rbc}$. To define this detection limit in terms of concentration, i.e. ppm, it is necessary to know the total volume of red cells in the original sample. In practice, this is, of course, measured after the the blood has been spun down. For now, assuming

a hematocrit of 0.36, i.e., in the "normal" range for children between 1 and 6 years of age, a 700 μl whole blood sample would yield $700 \times 0.36 \approx 250$ μl rbc. Therefore, at the limit of 0.065 μg Pb in the 120 μl sample, the 250 μl of red cells would contain $0.065 \times 250 / 120 \approx 0.14$ μg Pb. Thus, for the 700 μl whole blood sample with a "normal" hematocrit of 0.36, the 0.065 μg Pb detection limit is equivalent to 0.14 μg Pb / 0.7 ml ≈ 0.2 $\mu\text{g}/\text{ml}$. Referring to Table IV, it will be recalled that the counting time required to achieve a detection limit of 0.2 $\mu\text{g}/\text{ml}$ at the same significance level with the 400 μl whole blood sample was 2.5 min. Thus, the separation technique has now eliminated the need for hemoglobin-related background corrections, and the improved specimen geometry has shortened the time required to achieve the desired detection limit.

The Si(Li) detection used in the experiments to this point had an area 200 mm^2 with a resolution of 280 ev. As will be shown in the next section and subsequently in the description of the multi-element calibration, the use of a smaller 80 mm^2 detector resulted in a significant reduction in background in the Pb region as well as an improved resolution to 170 ev.

iii. Pb Calibration - 400 μ l whole blood/
60 μ l rbc/80 mm² detector

As will be shown, the increase in X-ray output realized with the improved specimen geometry was even more noticeable in the low energy Cl, K, Ca regions than in the Pb region. It was found subsequently that the low energy X-ray output could be still further increased with use of a smaller and hence less dense sample size, thus facilitation of the measurement of these elements. The smallest sample sizes that could be pressed and remain self-supporting and stable during handling were found to be 60 μ l of rbc's and 200 μ l of plasma, which were obtained from a whole blood volume of 400 μ l, again assuming hematocrits of 0.2 and 0.5. The average weights obtained with replicate samples of these volumes were 22 ± 1 mg and 21 ± 1 mg for the rbc's and plasma, respectively.

The Pb calibration factor obtained with this sample size was 170 cpm/ μ g Pb, significantly lower than that (360 cpm/ μ g) achieved with the 120 μ l rbc sample. This reduction in sensitivity was somewhat offset, however, by the reduction in background from 410 cpm to 105 cpm in the Pb region, due in part to the reduced scatter from the smaller

sample and also in large part due to the use at that point of a smaller detector (80 mm²) with improved resolution (170 ev) which allowed a much narrower region to be used for the Pb L_α peak. Even with large reduction in background, however, the reduction in sensitivity with the 400 μl sample size caused an increase in the detection limit of more than a factor two compared to the 700 μl size.

The Pb detection limit using these factors is calculated as before at the 0.05 significance level of a 2 min. counting time as follows:

$$(1.64 \sqrt{105/2}) / 170 \text{ cpm}/\mu\text{g} \approx 0.07 \mu\text{g Pb.}$$

Assuming a hematocrit of 0.36, the 400 μl sample gives a 144 μl volume of rbc's from which is taken the 60 μl sample. Thus, 0.07 μg Pb x 144/60 ≈ 0.07 μg Pb total and 0.12 μg/0.4 ml ≈ 0.42 μg Pb/ml.

The reduction in volume from 700 to 400 μl has thus resulted in an increase in detection limit from 0.20 to 0.42 μg/ml for a two-minute counting time, i.e. approximately a factor of 1.5 times the value which is considered the upper range of normal, as shown in Table I.

It is of interest to note that the detection limit expressed in terms of $\mu\text{g Pb/ml}$ whole blood is now a function of the hematocrit and/or hemoglobin content of the blood. Thus, given a hematocrit of 0.2, the 400 μl sample would give an 80 μl rbc volume and the 0.07 $\mu\text{g Pb}$ detection limit would have to come from a total of $0.05 \times 80/60 \approx 0.09 \mu\text{g Pb}$ in the whole sample, for a two-minute detection limit of $0.09 / 0.4 \approx 0.23 \mu\text{g Pb/ml}$ whole blood. The detection limit, therefore, improves as the red cell/hemoglobin content of the blood decreases below normal.

Table VIII summarizes the Pb calibration results using the rbc fraction and the truncated cone specimen shape.

TABLE VIII

<u>Sample Size</u>	<u>Detection Limit*</u>	
	<u>hematocrit = 0.36</u>	<u>hematocrit = 0.2</u>
700 μl w.b./120 μl rbc	0.20 μg Pb/ml w.b.	0.11 μg Pb/ml w.b.
400 μl w.b./ 60 μl rbc	0.42 μg Pb/ml w.b.	0.23 μg Pb/ml w.b.

*Calculated at 0.05 significance level for a 2-minute counting time.

IV. Multi-Element Analysis

As mentioned previously, one of the reasons for developing the technique for separation of the rbc and plasma fractions of the blood sample was to increase the sample output of fluorescent X-rays from the excitations of low Z elements present, to facilitate multi-element analysis. The real analytical power of the energy-dispersive XRF technique resides in the ability to simultaneously measure all of the elements present in the sample. Although in theory it is possible to measure all of the elements in the periodic table, in practice there is a limit at the low energy end due to absorption effects with a cut-off around Na ($Z = 11$). Our own system is limited by self-absorption effects due to sample thickness and additionally by detector window thickness to a low Z cut-off at Si. As seen from Table I, this would exclude the measurement of Si, F, Li, Na, and Al in blood using this technique and our current system. Rb would also be excluded because of its energy correspondence with the $U_{L\alpha}$ backscatter peak. The rest are measurable by excitation of either their K or L shell electrons. Because of the use of Au as additional shielding on the source causing spectral interferences, Au, Hg and Ag are not measurable in blood with this technique. Sn would also be very difficult to measure using L X-rays because of their proximity to the X-rays of potassium, which is present in such large amounts in blood in comparison to Sn. The same is true for iodine $L\alpha$ X-rays next to Ca_K X-rays. Thus, while it is not possible

to analyze all of the trace elements in blood simultaneously with our system, it would be possible to detect and measure the majority of them, viz., S, Cl, K, Ca, Cr, Mn, Fe, Cu, Zn and Br, in addition to Pb.

As mentioned previously, this would facilitate the use of the XRF technique in the possible correlation of fluctuations of these elements with disease states. Also, interest is increasing in the relationship of the concentrations of other elements in blood to the observed toxicity of a given concentration of Pb; e.g., there has long been evidence (16) that chronic Pb burdens are associated with anemia and depression of Fe levels because of interference of Pb in heme synthesis. There is also evidence (17,18,19) that lowered concentrations of Fe, Zn and Ca also affect Pb toxicity, probably by initially increasing lead uptake. The ability to measure these elements simultaneously in a sample of blood is therefore of importance and calibration of the system for the analysis of these elements was performed.

A. Signal to Background Improvement:

1. 700 μl sample size - 200 mm^2 detector: As indicated in Table VIII above, the change in specimen shape did not have a dramatic effect on the detection limit for Pb in blood for the 120 μl rbc fraction of the 700 μl sample, compared to the earlier 400 μl whole blood sample. As discussed previously, however, the new specimen geometry with the separation of rbc's and plasma did result in a substantial increase in the X-ray output of the lower Z elements by allowing an increase

in the sample surface area for excitation, while the sample weight, and hence areal density,* were decreased by approximately 40%, thus reducing absorption. The increase in signal to background realized as a result of these changes is shown in Figures 21 through 25. Figure 21 and Figure 22 compare the gross spectrum of the 76 mg whole blood sample and its weight-matched cellulose background in the trapezoidal shape to a 44 mg rbc spectrum with its weight-matched cellulose background, using the cone-shaped specimen geometry. The increase in the K region counts above background is substantial. These figures also illustrate how the fluorescence X-ray peaks of the sample are added to the background spectrum consisting of the backscatter peaks (only the $U_{L\alpha}$ and U_{L1} Compton scatter is shown), the low energy Compton continuum, and the extraneous peaks due to Ni and Au in the source housing. The net spectra of the whole blood, rbc and plasma, compared in Figures 23, 24, and 25, further illustrate the increase in signal in the Cl, K, Ca region.

The increase in signal to background achieved with the improved sample geometry in conjunction with the decrease in sample weight with fractionation led to the reduction in sample volume from 700 μl back to 400 μl to obtain the 22 mg samples used in in the multi-element calibration.

2. 400 μl sample - 80 mm² detector: Because of the possible importance of the measured concentrations of the

* Areal density is in units of gm/cm².

low Z elements such as Ca, it was decided to attempt to increase the signal from them by further reducing the volume of sample used, thereby further reducing the weight and the areal density. As mentioned previously, it was found that reproducible, self-supporting samples of the cross-section shown in Figure 16B could be achieved with a 60 μl rbc sample obtained from the fractionation of a 400 μl whole blood sample. Assuming a 0.2 hematocrit, a 400 μl sample yields 80 μl of rbc's. The use of a 60 μl sample of rbc's thus accounts for the possible losses when removing the plasma/white cell fraction. A fixed plasma volume of $0.5 \times 400 = 200$ μl allows, as before, the possibility of a blood sample with greater than the average hematocrit, just as the fixed 70 μl rbc volume allows for hematocrits much less than average.

The surface area of the sample is estimated to be 0.63 cm^2 . With a dry weight of 22 mg, as mentioned earlier, the areal density of these samples is $22/0.63 \approx 35$ mg/cm^2 . Although still not "thin" from the point of view of the absorption of the low Z elemental (S, Cl, Cu, etc.) X-ray energies, it represents a considerable reduction in absorption at these energies in comparison with the 76 mg whole blood sample, at a density of $76/0.63 \approx 120$ $\mu\text{P}/\text{cm}^2$, and with the previous 44 mg rbc sample with a density of $44/0.63 = 70$ mg/cm^2 . Although it would be desirable to use even thinner samples to avoid matrix absorption effects at these low X-ray energies, it will not be possible with the present

geometry, because samples of less weight than 22 mg cannot be made self-supporting. Even at 35 mg/cm^2 , however, it has been shown (20), at least in blood and other tissues of comparable composition, that the interelement effect of secondary fluorescence* is negligible at intermediate and high Z and amounts to an error of less than 2% in the low Z region of silicon/sulfur. Our data also indicate the predominant effect to be that of matrix absorption.

The improvement in signal to background for the low Z elements using the less dense samples may be seen by comparing Figure 26 and Figure 27, the gross spectra of 22 μg samples of rbc's and plasma respectively, by comparing peak heights with those of Figure 22 and Figure 25, which show the gross spectra of the heavier samples of the same materials. Also to be noted is the significant improvement in resolution obtained with the smaller (80 mm^2) Si(Li) detector, which has a resolution of 170 ev at 5.9 keV. The Cl, K and Ca K_α X-ray peaks are now clearly defined, as are the Fe K_α and Zn K_α , both of which are now separated from the Ni peaks of the source.

B. Multi-element Calibration Procedure:

The basic premise used in the calibration procedure is that it is a three step procedure in which: (1) the gross spectrum is first corrected for background; (2) the net

(*Secondary fluorescence is a phenomenon associated with the XRF elemental analysis wherein the X-rays produced by primary excitation of one element in a sample cause secondary excitation of other elements in the sample which have absorption edges below this element's X-ray energy. The net result is an increase in the X-ray output of the other elements above that expected by primary excitation. The phenomenon is not pronounced when elements are present in trace amounts, i.e., less than 1%, as in blood.)

spectrum is then corrected for peak overlaps and matrix effects (absorption and enhancement); and (3) the corrected counts for each element are compared to the counts obtained from known amounts of single-element standards excited in the same geometry as the multi-element unknowns.

1. Background Corrections: As seen earlier, if Figure 22 the sample elemental X-ray peaks, are added to the spectrum which consists of the peaks due to incoherent (Compton) and coherent scatter of the primary source X-rays from the sample matrix, the Compton continuum under the element peaks, and peaks such as the Ni and Au peak due to materials in the source. This is the background that must be subtracted from the unknown spectrum.

Background correction under the Pb peak in the case of the whole blood sample, it will be recalled, was complicated due to the variation with weight of the sample. This variation with weight, which was due to total scatter variation with sample weight, has been largely eliminated with the use of separate fixed amounts of rbc's. Nevertheless, it is still possible to produce errors with background subtraction if proper care is not taken to match the matrix of the "element-free" background material to that of the specimen. Two effects are produced by mismatch; spectrum shape and therefore background in the vicinity of the incoherent scatter peak can differ from the specimen because the amount of scatter is dependent on the effective Z of the sample. In the case of blood, this would effect the analysis of Br,

e.g., with its K_{α} peak between the U_{L1} and $U_{L\alpha}$ Compton scatter peaks; also, as will be demonstrated subsequently, the matrix is the primary absorber of the trace element X-rays and differences in absorption between single-element standards and the specimen matrices will cause count-rate errors in addition to background differences. Because of the large numbers of incoherently and coherently scattered X-rays, changes in their numbers also affects the size of the Ni and Au background peaks. The Compton continuum, which appears under the elemental peaks, is much less affected by matrix mismatch. The levels of the various trace-elements in the sample have a more marked influence on the shape of the spectrum here, as seen, e.g., in the difference in background level in the rbc and plasma spectra of Figures 26 and 27 below the area of the Fe peak. In this area however, which is free of any interferences due to peaks in the background spectrum, background correction can be accomplished by extrapolating under the peak from the linear region on either side of the peak or region of interest. Once the net elemental spectrum is obtained, peak overlap corrections are applied.

2. Peak Overlap Corrections. The correction for peak overlap is made with the use of simultaneous equations to solve for the contribution of counts to the peak region of a given element(s) by the presence of another element. This is due to spectral overlap and does not include secondary fluorescence contributions. The number of equations to be

solved depends on the number of overlapping spectral peaks. Examination of Figure 26 and Figure 27, rbc and plasma spectra, indicated that the spectral overlap occurred in three distinct regions, each of which would be corrected with the use of three simultaneous equations. The three regions and the peaks involved are thus Cl, K, Ca; Cr, Mn, Fe; and Ni, Cu, Zn. Pb and Br are affected only by scatter and are treated as individual peaks needing only background correction to obtain net counts.

The simultaneous equations used are of the form (21) shown below for Cl, K, Ca:

$$I_{MCl} = R_{Cl} \cdot I_{TCl} + C_{Cl/K} \cdot R_K \cdot I_{TK} + C_{Cl/Ca} \cdot R_{Ca} \cdot I_{TCa}$$

$$I_{MK} = C_{K/Cl} \cdot R_{Cl} \cdot I_{TCl} + R_K \cdot I_{TK} + C_{K/Ca} \cdot R_{Ca} \cdot I_{TCa}$$

$$I_{MCa} = C_{Ca/Cl} \cdot R_{Cl} \cdot I_{TCl} + C_{Ca/K} \cdot R_K \cdot I_{TK} + R_{Ca} \cdot I_{TCa}$$

in which:

I_{MCl} , I_{MK} , I_{MCa} = measured X-ray intensity in the Cl, K, and Ca regions of the unknown spectrum.

$C_{Cl/K}$, etc. or $C_{A/B}$ = measured contribution of element B to the intensity in region A, i.e., the ratio of the intensity in region A to the measured intensity in region B from a standard containing only element B.

The same standards used to obtain single element calibration factors also provided the desired contribution factors.

I_{TCl} , I_{TK} , I_{TCa} = the X-ray intensity of a known amount of element Cl, K, Ca in region Cl, K, Ca, i.e., the single element calibration factors.

R_{Cl} , R_K , R_{Ca} , = relative X-ray intensity; the intensity I_{MCl} or I_{MK} or I_{MCA} , in some matrix M divided by the intensity I_{TCl} , I_{TK} , I_{TCa} from a known amount of Cl, K, or Ca, e.g., $R_{Cl} = I_{MCl}/I_{TCl}$. R_{Cl} , R_K , and R_{Ca} are the unknowns to be solved for in the three equations.

3. Single-Element Standards: The individual calibration factors from the single element standards were obtained by placing a known amount of the element, in solution, onto a 25 mm diameter, type HA, Millipore cellulose-base filter paper. After drying under a heat lamp, the filter paper was pressed into a cone-shaped sample for analysis. Calibration factors for each of the elements Cl, K, Ca, Cr, Mn, Fe, Ni, Zn, Br and Pb were obtained from the mean of three filter standards of each element at three different concentrations. This procedure was used to isolate possible calibration errors due to incorrect weighing of standards or differences due to uneven distribution of the dried standard on its filter paper base.

The results of the single element calibrations in cpm/ μ g vs. Z are shown as the crosses by the dashed line in Figure 28.

Using the contribution factors obtained from single-element standards, the solution to the three simultaneous equations for the Cl, K, Ca region resulted in the following equations for R_{Cl} , R_K , and R_{Ca} :

$$R_{Cl} = [I_{MCl} - 0.0099 I_{MK} - 0.006 I_{MCA}] / I_{TCl}$$

$$R_K = [I_{MK} - 0.0184 I_{MCA} + 0.006 I_{MCl}] / I_{TK}$$

$$R_{Ca} = [I_{MCA} - 0.141 I_{MK} + 0.0099 I_{MCl}] / I_{TCa}$$

The largest corrections are thus for the contribution of counts to K by Ca and Ca from K. The contribution to K or Ca by Cl, or vice versa, is small since the Cl peaks (K_α and K_β) are fairly well separated from the K and Ca region as seen in the rbc and plasma spectra of Figure 25 and Figure 26.

4. Comparison with Orchard Leaf and Bovine Liver Standards:

To test the accuracy of the equation for the nine elements involved, as well as for Pb and Br, samples were pressed from 22 mg aliquots of NBS standard orchard leaves and bovine liver which contain known amounts of most of the elements used in the calibration procedure.

The spectra of these standard materials are shown in Figure 29 and Figure 30. The background-corrected spectrum for these samples was obtained by subtracting the spectrum of a sample pressed from powdered cellulose acetate. This material provided a better fit to these standard spectra than did the filter paper, especially in the region of the Compton and Rayleigh scatter energies.

a) Orchard Leaf Absorption Correction: After subtraction of the background, the matrix counts thus obtained for the orchard leaf standard were corrected for overlap in each of the three regions to obtain the corrected net matrix counts I_M . The net matrix counts divided by the net intensity

I_T , obtained from the single element filter-paper standards counted in the same geometry, yields the desired ratio $R_i = I_{Mi}/I_T$ for any element, i , in the sample. Multiplying these ratios by the known amount of the element on the filter-paper standard should yield the amount of the element in the sample.

The concentrations thus calculated for the orchard leaf standards were found to be too high for the elements Cl, K and Ca, indicating a possible systematic error in the calibrations. Also, because of the improvement in fit seen in the Compton/Rayleigh scatter regions of the orchard leaf spectra using a cellulose-acetate background as opposed to the filter paper, matrix absorption differences between the orchard leaf sample and filter paper standards were suspected. As discussed earlier, the ratio of the Compton-Rayleigh scatter intensities is a sensitive index of the ratio of low Z and high Z element content of the matrix and has been used (22) as a predictor of the average Z of an unknown matrix.

To check this possibility, calibration factors were calculated from the net orchard leaf results using the known amounts of the elements contained in the orchard leaf sample, and assuming that the contribution factors utilized for the overlapped peak regions obtained from the filter-paper standards would remain valid.

The calibration curve resulting from these calculations is shown in Figure 28, where it is seen that the values thus

obtained from the orchard leaf sample (solid line) are in fact higher than those obtained with the single-element filter paper standards (dashed curve) below the Fe region ($Z=26$). The error bars on Br point indicate that there may not be as much difference as shown in that region since only counting errors are included. The points for Cl and Cu are also seen to be off the smooth curve drawn through the other points for the single-element standards, indicating possible errors in the filter paper standard dilutions for these elements as well as Br. Not shown in the figure is the filter-paper standard value for Pb ($Z=82$) which was 168 cpm/ μg compared to the 170 cpm/ μg obtained with the orchard leaf standard.

2) Bovine Liver Results: The orchard leaf calibration factors were now checked by using them to determine the concentration of these elements in bovine liver. The results obtained are shown in Table IX and compared to the known NBS values for these elements. The agreement is good for all the elements and the differences seen probably reflect, in addition, to counting error, errors in the contribution factors and differences in the concentration of the low Z matrix elements, i.e., H, C, O, N, between orchard leaf and bovine liver. As stated earlier, secondary fluorescence effects, which are not accounted for in the results, have been shown to be small

TABLE IX

<u>Element</u>	<u>Bovine Liver Concentrations ($\mu\text{g/gm}$)</u>	
	<u>XRF^a</u>	<u>NBS^b</u>
S	7588 $\pm$ 1400	--
Cl	2638 $\pm$ 280	(2600)
K	8992 $\pm$ 800	9700
Ca	128 $\pm$ 6	(123)
Mn	7.7 $\pm$ 2	10.3
Fe	299 $\pm$ 10	270
Cu	177 $\pm$ 20	193
Zn	138 $\pm$ 7	130
Br	9.8 $\pm$ 1	--
Pb	< 0.5 ^c	0.34

^aError shown is two standard deviations and includes the counting errors of both the bovine liver and orchard leaf calibration factors.

^bValues in parentheses are not certified by NBS. S and Br values are not reported.

^cDetection Limit (0.05 significance level) for this element in 22 mg bovine liver sample, with 30 min. counting time.

from these material. The differences between the calibration factors obtained above between filter paper and orchard leaf standards serves to illustrate the necessity to match the matrix of the standard to the matrix of the sample as closely as possible.

Since the chemical makeup of most biological materials is probably known, at least for most tissues, it should be possible to match background and standard matrix material to sample material within reasonable tolerances so only small corrections, if any, may be necessary.

An example of this would be the rbc fraction of blood. Since rbc's contain greater than 95% hemoglobin, this becomes the matrix to match. The chemical formula for hemoglobin according to the Merck Index is:

The composition of hemoglobin is shown in Table X along with the composition of Millipore type H.A. filter paper, cellulose acetate, and cellulose acetate with boric acid. Directly below the composition are values of the absorption coefficient of these materials versus energy, calculated using their known composition. As seen, the composition of the millipore filter paper is quite different from hemoglobin. Even though composed partly of cellulose acetate, which is a better match of hemoglobin, the filter paper has 7 parts cellulose nitrate which

TABLE X
ELEMENTAL PERCENTAGES OF HEMOGLOBIN
AND OTHER MATERIALS

<u>Element</u>	<u>Hemoglobin</u>	<u>Cellulose Acetate</u>	<u>Millipore Paper</u>	<u>Cellulose Ace. & Boric Acid</u>
C	54.3%	50.0	31.1	53.8
O	20.4%	44.4	55.2	37.7
N	17.4%	-	10.5	-
H	7.2%	5.6	3.2	5.9
S	0.39%	-	-	-
Fe	0.34%	-	-	B = 2.5

μ/ρ (cm²/g) OF HEMOGLOBIN AND
OTHER MATERIALS vs ENERGY

<u>Energy</u>	<u>Hemoglobin</u>	<u>Cellulose Acetate</u>	<u>Millipore Paper</u>	<u>Cellulose Ace. & Boric Acid</u>
2 keV	392.54	455.76	523.16	407.99
4 keV	52.81	59.49	68.81	52.82
6 keV	15.22	17.23	20.03	15.23
8 keV	6.99	6.85	8.00	6.03
10 keV	3.52	3.36	3.92	2.96
15	1.00	0.92	1.09	0.81

overbalances the number of oxygens, resulting in an absorption coefficient approximately 30% greater than hemoglobin throughout most of the energy range shown. Assuming hemoglobin matches bovine liver in terms of its matrix, this in large part explains the differences in calibration factors seen using the filter paper standards.

The last column shows the composition and absorption coefficients of 50% cellulose acetate and 50% boric acid, a material commonly used in pelletizing XRF samples. While not a perfect match for hemoglobin, it comes much closer than cellulose acetate alone, especially in the 2, 4 and 6 keV energy range where the absorption errors are larger. It should be possible, with the addition of a compound such as LiNH_2 (Lithium Amide), to increase the absorption coefficient at higher energies while keeping the 2, 4, 6 keV range approximately the same.

In this manner, the matrix of hemoglobin, i.e., rbc's and other biomatrices may be matched to other trace element-free, organic matrices using absorption coefficient versus energy to guide matrix composition and thus keep background and absorption corrections small.

V. Discussion

The primary purpose of the work performed under this contract, as stated in the introduction, has been the development of a system for the detection of Pb in blood utilizing radioisotope excited XRF analysis. For practical applications, the goal is a system which will measure "normal" levels, i.e., $< 0.3 \mu\text{g/ml}$ (0.3 ppm, 30 $\mu\text{g/dl}$), of Pb in an amount of blood obtainable with a finger-stick or other sampling method (e.g., ear lobe puncture) requiring as little sample preparation as possible, with as short an analysis time as possible.

For purposes of defining a detection limit of $< 0.3 \mu\text{g/ml}$, a working goal of $0.2 \mu\text{g/ml}$ has been used throughout this report. The results presented indicate that this detection limit can be achieved, however, not without a compromise in one or another of the contingent prerequisites, i.e., sample size, ease of sample preparation, or analysis time.

Thus, from the data presented in Tables V and VI, it is seen that shortest analysis time to obtain the $0.2 \mu\text{g/ml}$ limit is achieved using the 700 μl whole blood sample size with the separation technique and a 120 μl rbc aliquot for the analysis. Although the 400 μl whole blood sample without separation has approximately the same sensitivity and detection limit (assuming a "normal"

hematocrit - 0.2 $\mu\text{g}/\text{ml}$; 2.5 min.) with the least complicated sample preparation, the calibration errors which arise as a result of variation of background and sensitivity with sample hemoglobin content make this procedure, even with its simplicity, less favorable than the one including the centrifugation/separation steps. These steps result in samples of constant weight and thus yield the statistically predictable scatter background and sensitivity needed for measurements which will be made at or near the limits of detection. Unfortunately, the 400 μl sample using the separation technique and a 60 μl rbc specimen resulted in a significant loss in signal from the 10.5 keV X ray of Pb. Although the smaller specimen size did improve the signal to background ratio in the low Z region, use of this sample size for the detection of Pb would require an 8 min. counting time to reach the 0.2 $\mu\text{g}/\text{ml}$ detection limit - a factor of 4 greater than the 700 μl sample size.

Thus, it may be concluded that for purposes of measuring the concentration of Pb in blood at normal levels, i.e., < 0.3 $\mu\text{g}/\text{ml}$, the 700 μl sample size with the separation procedure gives the best results. People who draw blood routinely assure us that this amount of blood and more, is easily obtainable with a vigorous finger-stick procedure. Moreover, the possible error due to external contamination is lessened considerably with the larger sample size.

It will be recalled that the value of 0.2 $\mu\text{g}/\text{m}\ell$ for the detection limit obtained with the 700 $\mu\ell$ sample size was calculated using a K_α of 1.64 as follows:

$$\text{Detection Limit} = K_\alpha \cdot \sqrt{\text{bkgd}/t_b}$$

where $\sqrt{\text{bkgd}/t_b}$ is the standard deviation of a Pb-free background sample counted for a time t_b . For the 42 mg, 120 $\mu\ell$ rbc sample with a background of 410 cpm, the standard deviation for a 2 min. count is thus $\sqrt{410/2} = 14.3$ cpm. K_α , taken from the standardized normal distribution, is set by the α or Type I error selected. A Type I error is made by deciding that Pb is present in the sample when, in fact, it is not. In this case, a value of $K_\alpha = 1.64$ represents a significance level of 0.05, which is the probability that a sample containing no Pb will have a count rate that exceeds $1.64 \sqrt{410/2} = 23.5$ cpm. Therefore, for any given sample to be considered positive, i.e., to say Pb is present, it must have a net count rate of 23.5 cpm or more. An α level of 0.05 thus represents the probability of making a false positive statement - saying lead is present when it is not. Dividing the critical level of 23.5 cpm by the calibration factor, 360 cpm/ μg Pb thus yields the detection limit of $23.5/360 \approx 0.065$ μg which translates into 0.2 $\mu\text{g}/\text{m}\ell$ whole blood assuming a normal hematocrit of 0.36.

For the detection of Pb, while false positives would be a nuisance at the working level of 0.2 $\mu\text{g}/\text{m}\ell$, this is well below the 0.3 $\mu\text{g}/\text{m}\ell$ considered to be an elevated Pb level and the probability of having a false positive at this level for a 2 min. count is 0.0055 ($K_\alpha = 2.56$). More important in the application of any device for the measurement of Pb is the probability of making a Type II error, i.e., saying Pb is not present in a sample when, in fact, it is, or, recalling Table I, putting a child with a given positive blood sample into a lower risk category than he might actually belong in. Considering the relatively small increments between these categories, it is desirable to set the probability of making the Type II or B error, i.e., a false negative, as low as possible with the limitations of the test procedure.

As before, the variable K_β is selected from the standardized normal distribution and used with K_α to determine the critical level,

$$\beta \text{ detection limit} = (K_\alpha + K_\beta) \sqrt{410/2}$$

For $K_\alpha = 1.64$ ($\alpha=0.05$) and $K_\beta = 1.96$ ($\beta=0.025$), the β detection limit is 0.43 $\mu\text{g}/\text{m}\ell$. This is clearly too high since a β significance level of 0.025 means that a sample containing this amount of Pb could be expected to yield a

count rate less than the K_α level, i.e., less than background, 2.5 times out of 100.

It would be desirable to decrease the critical level for the Type II error to $0.3 \mu\text{g/ml}$, i.e., at the bottom of the moderate risk category of Table I, thus making the probability of the Type II error small for children actually in this category. To do this with the K_α and K_β values above ($K_\alpha = 1.64/\alpha = 0.05$; $K_\beta = 1.96/\beta = 0.025$) requires an increase in counting time to 4 minutes. Thus:

$$(1.64 + 1.96)\sqrt{410/4} \text{ cpm}/360 \text{ cpm}/\mu\text{g} = 0.1 \mu\text{g Pb}$$

which converts to $0.3 \mu\text{g/ml}$ whole blood. With this counting time, it can be shown also that the high risk (0.5 to $0.69 \mu\text{g/ml}$) and critical risk ($> 0.7 \mu\text{g/ml}$) categories have even lower β errors. Thus, the probability of a $0.7 \mu\text{g/ml}$ level falling into the moderate risk category is kept to 0.01. Considering the importance of the various risk categories, the increase in counting time to 4 minutes is not overconservative.

Comparing the XRF technique developed here to the Anodic-Stripping Voltometry method discussed in the introduction - the only other transportable method with a sample preparation requiring no chemistry - it is seen that, in terms of time, the ASV method, capable of 30 Pb analyses/hr, is faster than the XRF method with the 4 min. counting time requirement. As has been shown,

however, the real power of the XRF method lies in the ability to analyze, simultaneously, the levels of other elements in blood, including Fe, Ca and Zn which have been found to be indicators of the toxicity of a given Pb burden. The ability to measure these other elements simultaneously with Pb more than offsets the somewhat slower analysis time.

It is of interest to note here that the ability to measure these other elements simultaneously may be useful in expressing the results of the Pb measurements in terms that would reflect in a more definitive way the toxicity of a given level of Pb in blood. For example, by measuring the amount of Fe present with Pb, the results could be expressed as $\mu\text{g Pb/gm Fe}$ in addition to the usual $\mu\text{g Pb/ml whole blood}$. When measured by other methods, a blood level of $0.3 \mu\text{g/ml}$, considered only slightly elevated, would give the same result regardless of hematocrit. $0.3 \mu\text{g/ml}$, however, in a 0.7 ml sample contains $0.21 \mu\text{g Pb}$, and, with a "normal" hematocrit of 0.36 contains approximately $236 \mu\text{g Fe}$ (8). Thus, with the inclusion of Fe, $0.3 \mu\text{g/ml}$ in "normal" blood would be expressed as $0.21/236 \times 10^{-6} = 734 \mu\text{g Pb/gm Fe}$, while the same Pb burden in blood with a hematocrit of 0.2 , containing approximately $158 \mu\text{g Fe}$, would result in a value of $0.21/158 \times 10^{-6} = 1329 \mu\text{g Pb/gm Fe}$.

Expressed in this manner, it may be possible that blood Pb concentrations measured and correlated with the measurements of erythrocyte porphyrin may produce a better expression of the toxic potential of a given Pb level than the EP test alone.

VI. Summary

This report has described the most significant experiments and the results obtained, during the development of a system for the detection and measurement of Pb in blood using radioisotope-excited X-ray fluorescence analysis, over the contract period. Briefly, the report described:

- 1) Detector Selection: A Si(Li) detector was selected for its excellent resolution characteristics. Based on the projected resolution required for the separation of the Pb L_{α} peak from backscatter and the X rays of other elements present in blood, a 200 mm² detector was selected having a resolution of 280 ev at 5.9 keV. Subsequently, an 80 mm² detector with a resolution of 170 ev was obtained. The larger area was selected first to maximize geometric efficiency. Because of source shielding requirements, however, this could not be taken advantage of in the final geometrical arrangement between sample and detector.
- 2) Source Selection: Pu-238, which emits U-L-X rays with energies close to the L absorption edges of Pb was chosen as the primary photon source. The L-X rays of Pb were used to avoid the overlap of K-X rays with the backscatter energies of Co-57

or Cd-109, the other radioisotopes that could be used to ionize the Pb atom. Although the Ag X ray emitted during the decay of Cd-109 also could have been used to excite the L-X rays of Pb, the cost of this isotope was prohibitive, in addition to its short half-life (< 1 year), in comparison to Pu-238 (460 years).

- 3) Source-Sample-Detector Geometry: To achieve maximum geometric efficiency a new source holder was designed which placed the source, in the form of microspheres, in a circle 2 mm in diameter between two collimating plates, each 4 mm in diameter (Figure 5). The blood sample, in the form of a toroid having an i.d. of approximately 4.2 mm and an o.d. of about 9 mm was placed around the source, which was placed on the window of the detector. In this manner, the tightest possible source-sample-detector geometry was achieved. Unfortunately, due to primary scatter off, the source and the sample surfaces, the source-sample plane had to be raised off the window and a piece of Au machined to fit the bottom collimator plate to reduce dead time and resolution losses. Three source-holder collimators were made and tested

during the course of the project; a 20 mCi source (Fig. 8) with five 1 mm diameter beads, a 25 mCi source using 25 microspheres of Pu-238, 250 μ in diameter, a 50 mCi source (Fig. 15) made with the source material in the form of "shards".

- 4) Refinement of Sample Preparation: The sample preparation technique went through several stages in the quest to achieve a stable, reproducible, self-supporting sample in the best geometrical configuration; from a toroid of rectangular cross section (Fig. 15) cut from absorbed material into which the 150 μ blood sample was placed for drying under a heat lamp, through a self-supporting toroid sample with a trapezoidal cross section (Fig. 16A) with a weight of 76 mg, formed by pressing a 400 μ l whole blood sample in a specially machined steel jig after drying in a microwave oven. This was followed by a sample in the shape of a doubly-truncated cone (Figure 16B) when it was found that separation of the whole blood into rbc and plasma fractions was required to obtain samples of constant weight. In its final form, the sample would consist of 60 μ l of rbc's and 200 μ l of plasma

dried to a weight of 22 mg from a 400 μl sample.

- 5) System Calibration: Intercomparison differences between XRF and A.A. noted during the calibration of the system using the 400 μl , 76 mg samples was traced to weight variations which were subsequently found to be directly proportional to the hematocrit and/or hemoglobin content of the blood sample. Calibration experiments were then performed to take these variations into account and showed that the background count in the Pb region of a Pb-free sample of blood ranges from 360 to 500 cpm for sample weights between 58 mg and 93 mg. Over the same weight range the sensitivity varied from approximately 100 to 80 cpm/ppm. Using the calibration curves thus developed, subsequent intercomparison measurements showed good agreement between XRF and A.A. in measurements of Pb in baboon blood as well as children's blood samples obtained from New York City.
- 6) Separation Technique: To obtain a constant sample weight and avoid the calibration problems noted above, it was decided to add a centrifugation step in the sample preparation technique.

By selecting a constant volume of rbc's based on the lowest hematocrit seen (0.2), a constant sample weight and hence constant background and constant sensitivity factor could be achieved, regardless of the hemoglobin content of the sample. Fractionation would also facilitate the measurement of elements concentrated in one or the other fraction, as e.g. K, Fe and Pb are in the rbc fraction. A change in the sample shape to a truncated cone at this stage (Fig. 16B) was shown to increase the signal-to-background ratio for the low Z elements Cl, K and Ca in either fraction. A 700 μl sample size was used to obtain a rbc sample of 120 μl and a plasma sample of 350 μl . This was subsequently changed to a sample size of 400 μl to give rbc and plasma samples of 60 μl and 200 μl respectively and a constant sample weight of 22 ± 1 mg. The smaller sample size, and hence density, decreased the absorption in the Cl, K, Ca regions resulting in another increase in signal to noise. This was done to facilitate the simultaneous measurement of these and other elements in the sample along with Pb.

7) Multielement Calibration: Because the real power of energy-dispersive XRF analysis is the ability to simultaneously measure all of the elements in a sample and because other elements such as Fe and Ca were shown to be important as possible indicators of the toxicity of a given Pb burden, a multi-element calibration was sought to test the test of the utility of the system in this manner. The calibration was facilitated with the acquisition of a 80 mm² detection with a resolution of 170 ev compared to the previous 200 mm², 280 ev detector. By separating the spectrum into 3 groups of 3 elements each, viz, (Cl, K, Ca), (Cr, Mn, Fe), and (Ni, Cu, Zn) it was possible to account for spectral overlap of K_α and K_β peaks with the use of 3 simultaneous equations in each region. The contribution factors for these equations as well as the calibration factors for all the elements including Pb and Br were obtained by placing known amounts of these elements on Millipore filter paper, drying, and pressing them into the sample shape. The filter paper had been found to be a reasonable background match for the blood samples. When

these calibration factors were applied to the net areas under the peaks of a sample of NBS orchard leaves, the values were found to be in error. By using the known concentrations of the calibration elements present in orchard leaves, a second calibration curve was constructed (Fig. 28). The differences were subsequently found due to differences in absorption coefficients between the filter paper and the sample. The orchard leaf calibration coefficients were used to measure the concentrations of these elements in a sample of NBS bovine liver and gave acceptable values compared to the NBS values for that material. Unfortunately, the reduction sample density reduced the Pb calibration factor from 360 cpm/ μg to 170 cpm/ μg . Background, as a result of reduced scattering, and improved resolution was also reduced to 105 cpm, and although the less dense sample improved the signal-to-background ratio for the low Z elements, the counting time to reach a detection limit of 0.2 $\mu\text{g}/\text{ml}$ was increased to 8 min., as compared to the 700 μl sample size which can achieve the same limit with a 2 min. counting time.

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Figure 1. Mass Attenuation Coefficient of Lead vs Energy

Figure 2

Center Source Geometry

Annular Source Geometry

Figure 3

Figure 4. The number of photons scattered into unit solid angle $d(\epsilon_\sigma)/d\Omega$, at a mean scattering angle ϕ .

FIGURE 5

Section A-A

(All dimensions are in millimeters)

Figure 6

Section B-B

Figure 7

FIGURE 8

Figure 10.
Detector Energy Calibration Curve

Figure II. Source from the Side

Figure 12.

Key to Figure 12

- A. Chlorine- K_{α} (2.62 keV)
- B. Potassium- K_{α} (3.3 keV)
- C. Potassium- K_{β} (3.6 keV)
- D. Chromium- K_{α} (5.4 keV)
- E. Iron- K_{α} (6.4 keV)
- F. Iron- K_{β} (7.06 keV)
- G. Copper- K_{α} (8.04 keV)
- H. Copper- K_{β} (8.9 keV)
- I. Gold- L_{α} (9.7 keV)
- J. Lead- L_{α} region
- K. 180° scatter
- L. 11.3 keV compton 90° scatter
- M. Gold- L_{β} (11.5 keV) + Uranium L_{α} scatter (11.3 keV)
- N. Bromine- K_{α} (11.9 keV)
- O. 180° scatter
- P. 13.3 keV compton 90° scatter
- Q. 13.6 keV coherent scatter
- R. 180° scatter
- S. 15.9 keV compton 90° scatter
- T. 180° scatter
- U. 16.6 keV compton 90° scatter
- V. 17.2 keV coherent scatter

} Uranium- L_{α}

} Uranium- L_{α}

} Uranium- L_{β_2}

} Uranium- L_{β_1}

Figure 13. Source, Copper face down

Figure 14. Lead Calibration Curve

Optimized Source Design

(All dimensions are in millimeters)

FIGURE 15

FIGURE 16A

FIGURE 16B

FIGURE 17

FIGURE 18

Count Rate in Pb Channel vs. Total Pb in Sample

FIGURE 19

FIGURE 20

FIGURE 21: 76 mc Whole Blood vs.
76 mc Cellulose Background,
Expanded

FIGURE 22: RBC vs. Cellulose
Background

-125-

FIGURE 23: Whole Blood Net Spectrum

-126-

FIGURE 24: RBC Net Spectrum

-127-

.00

1/P1

CL = 2077 LT = 1000

4096FS

13.64

FIGURE 25: Gross Plasma vs. Net Plasma

-128-

FIGURE 26: 22 mg RBC Sample Spectrum.

FIGURE 27

I-4 Plasma

FIGURE 28

FIGURE 29

FIGURE 30

IX. Appendix A

Procedure used for separation of 700 μl volume of whole blood into rbc and plasma fractions and preparation of dried toroidal samples.

- 1) Using a Pasteur pipette, fill Wintrobe tube to the 100 mm mark with whole blood that has been gently and thoroughly mixed.
- 2) Spin blood for 30 min. at 8000 rpm (10,400 g).
- 3) Record:
 - a) total volume mark, i.e., 100 mm
 - b) rbc/plasma interface mark
 - c) hematocrit = KBC volume/total volume
- 4) Withdraw approximately 353 μl plasma with Pasteur pipette. Wintrobe tube i.d. = 3 mm; volume = $7.0686 \text{ mm}^3/\text{mm}$ of length and 100 mm mark = 707 μl . Withdraw plasma until remaining Wintrobe volume = total volume - 50 mm. $50 \text{ mm} \times 7.0686 \text{ mm}^3/\text{mm} \approx 353 \text{ } \mu\text{l}$ plasma.
- 5) Put plasma sample on teflon disk and place in microwave oven for 3 min. Remove remaining plasma (including white cell interface) with same pipette and discard.
- 6) Withdraw 127 μl rbc's. Because of the viscosity of the red cells, a 300 μl capillary tube (154.5 mm length) with a suitable suction apparatus is used in place of the Pasteur pipette. Insert the

capillary tube to the bottom of the Wintrobe tube and fill it to the 65 mm mark as observed on the Wintrobe tube scale. Thus, $65/154.5 \times 300 = 127 \mu\text{l}$ is withdrawn from the Wintrobe in the capillary tube.

- 7) Deposit rbc's on teflon disk and place in microwave oven for 5 min.
- 8) Press dried rbc and plasma into toroidal shapes.

The final version of the steel jig used to press the sample is shown in cross-section in Figure 1 below. In practice, pieces A, B, and C are joined together and the dried specimen is placed in the "space" formed by the intersection of the shaft A, the indent at the top of B, and the inner wall of C. The cap, D, is then inserted in C and the sample is hand-pressed to initially compress the sample after which 5-10 μl of alcohol is dropped on the sample to wet it. The cap is then replaced and the jig placed in a hand-held KBR press. After removal from the press, cap D is removed from the jig followed by piece A which is taken out with a gentle, twisting movement. The sample, now pressed into shape, is on top of piece B within piece C. A teflon shaft 9 mm in diameter (not shown in the figure) is used to push piece B out of piece D. The sample is now removable from piece B using a sharp-edged tool such as a scalpel at the interface to lift it out.

X. Appendix B

The work described in this appendix, reported in the form of a Master's degree thesis, is presented in this report to illustrate the usefulness of the source design and sample preparation technique for the measurement of trace elements in a variety of matrices, even though the methodology was developed specifically for blood analysis.

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