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# Studies of Aluminum in Rat Brain

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Running title: Aluminum in Brain

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**ABSTRACT**

The effects of high aluminum concentrations in rat brains were studied using $^{14}C$ autoradiography to measure the uptake of $^{14}C$ 2-deoxy-D-glucose ($^{14}C$-2DG) and microbeam proton-induced x-ray emission (microPIXE) with a 20-μm resolution to measure concentrations of magnesium, aluminum, potassium, and calcium. The aluminum was introduced intracisternally in the form of aluminum tartrate (Al-T) while control animals were given sodium tartrate (Na-T). The $^{14}C$ was administered intravenously. The animals receiving Al-T developed seizure disorders and had pathological changes that included cerebral cortical atrophy. The results showed that there was a decreased uptake of $^{14}C$-2DG in cortical regions in which increased aluminum levels were measured, i.e., there is a correlation between the aluminum in the rat brain and decreased brain glucose metabolism. A minimum detection limit of about 16 ppm (mass fraction) or $3 \times 10^9$ Al atoms was obtained for Al under the conditions employed.

Index Entries: PIXE, Aluminum, Autoradiography, Encephalopathy, Aluminum toxicity, $^{14}C$ 2-deoxy-D-glucose

## INTRODUCTION

Interest in the health consequences of increased levels of aluminum in the brain arises from several sources. Dementia has been documented in uremic patients who received large doses of antacids containing Al (1) and were dialyzed with water which contained increased levels of aluminum. Increased Al levels in brain tissue have been demonstrated in patients with Alzheimer's Disease and in Guamanian natives with a Parkinsonian-dementia diagnosis (2). It has also been observed in many laboratories that human subjects with Alzheimer's Disease have decreased brain glucose utilization (BGU) using PETT imaging techniques. The PETT technique employed in clinical studies, which utilizes $^{18}$F-2DG as the BGU tracer, measures the convolution of blood flow, membrane transport, and hexokinase activity in the neurones perfused by blood containing the intravenously-injected tracer.

The affinity of hexokinase for Al is several orders of magnitude greater than for Mg, its natural cofactor (3). This observation led to the development of an animal model (rat) for further study, using large doses of Al injected into the cerebral-spinal fluid (CSF) (4). Rats given these large doses developed EEG evidence of seizure activity approximately 19 days following Al injection, and died sometime in the next week, at which time histopathological evidence of degenerative changes was found in the cerebral cortex.

Progress in understanding the biological role and the biological pathways taken by aluminum as it enters the brain has been slowed by difficulties in analyzing small tissue sections for minuscule numbers of aluminum atoms. Perl et al. observed aluminum in human brains using a scanning electron microscope (SEM) with an energy-dispersive x-ray detector (5,6). Garutto et al. refined

the earlier experiment and used a SEM with both energy- and wavelength-dispersive detectors (7). They estimated calcium and aluminum concentrations in one neurofibrillary tangle-bearing neuron of 7200 and 500 parts per million (ppm) mass fraction, respectively. High sensitivity, that is, values in the ppm range, are difficult to obtain with SEM XRF because of the bremsstrahlung background. Perl (8) has more recently used a Laser Microprobe Mass Analysis (LAMMA) to carry out the same analysis. In this case the sensitivity is in the ppm range, but accurate quantitative values are difficult to obtain because of the sputtering-type process used to produce the ions to be analyzed.

A pilot study was undertaken to determine the feasibility of correlating changes in brain elemental content (microPIXE) and cerebral glucose metabolic changes from autoradiography (ARG). The specific aims of these studies were 1) to correlate Al distribution changes with glucose utilization measured by quantitative autoradiography, 2) to determine the microlocalization of Al in the rat encephalopathy model, and 3) to determine the minimum detection limits for Al in rat brain using small proton beams for proton-induced x-ray emission (microPIXE studies).

The use of microPIXE could be an important method for simultaneous measurement of the distributions of aluminum and heavier elements in the brain. Resolutions of about 1 μm can be attained in some instruments, and the minimum detectable limit (MDL) is in the ppm range. The sensitivity is better than for the electron microprobe since the background from proton bremsstrahlung is much less than the background from electron bremsstrahlung in the electron microprobe. For scanning purposes lower resolutions can be useful so that we were encouraged to proceed with the use of the Brookhaven

microprobe which is a medium resolution device capable of producing 20-μm diameter spot size beams.

An additional important point to emphasize is that for the analysis of tissue sections of the order of 5 to 30 μm thickness, scattering of the electrons gives effective beam diameters which may be larger and not appreciably better than those for the proton probe. Under these conditions, a specimen with a total mass of $2 \times 10^{-9}$ g under the beam will have an aluminum content at the 500-ppm (μg/g) level of only $10^{-12}$ g or $2 \times 10^{10}$ atoms. It can be seen that the resulting aluminum detection problem using either proton or electron probes is difficult at low concentration levels and small sample sizes.

**METHODS**

Sprague-Dawley rats weighing 300-350 grams were operated upon under pentobarbital anesthetic to insert EEG leads, and a cannula plate which was inserted to permit injections into the cerebral spinal fluid space (CSF). The animal model preparations were conducted at Vanderbilt University. The animals were given 7-14 days to recover, following which baseline EEG recordings were made. Half the animals were then given aluminum-tartrate (1.9 mg/20 μl) and half were given sodium tartrate (1.9 mg/20 μl) intracisternally. The latter group served as controls. Fifteen to 19 days later, the animals were brought to BNL where they were given 1-3 μCi of $^{14}$C-2DG intravenously. These animals were sacrificed 45 minutes later and prepared for ARG. Thin sections were cut (30 μm) using an LKB cryomicrotome, the sections were dried, powdered, and applied to film, which was then exposed for 15 days, along with reference standards. The $^{14}$C-2DG was quantitated for

individual pixels (50 μm × 50 μm) in selected regions of the brain section using methods presented previously (9). The same sections were taken to the Brookhaven Research Van de Graaff Accelerator for elemental analysis of 20-μm diameter areas in the same regions measured in the autoradiography.

Commercial standards were used for the $^{14}C$ quantitation (10). Aluminum standards for the microPIXE work were prepared by taking a 10 mg/ml stock solution of Al-tartrate in 20% HCl. This was diluted and mixed with polyethylene glycol to achieve concentrations ranging from 0 to 1000 ppm. Care was taken in the choice of reagents and glassware to minimize the possibility of contamination. The standards were frozen and sectioned (30 μm) on the same cryomicrotome used for the tissue samples.

The $^{14}C$-2DG was measured in multiple 50 μm × 50 μm cortical subregions encompassing the 20 μm diameter region in which the PIXE determinations were made. Data were analyzed using a quantitative videodensitometry system (9), and graded amounts of $^{14}C$ in commercial standards (10). Analysis of elemental content was based on measurements of area under each peak and reference standards in appropriate matrix geometries.

The beam line and sample irradiation geometry for the proton microprobe at the BNL 3.5-MV Van de Graaff Accelerator are shown schematically in Figure 1. A series of collimators and an electrostatic quadrupole lens (11) reduce the beam diameter from 100 μm at the object slits to 20 μm at the sample. The beam hitting the sample is monitored with an annular surface barrier detector at an angle of 165° to the incident beam. The alignment of the beam and the sample was effectuated using a microscope at a magnification of 200 X. The beam position was found relative to the microscope eyepiece cross hairs using a scintillator and the sample then positioned

appropriately. The microscope viewed the scintillator and target in the direction opposite to that of the incident beam. An energy-dispersive Si(Li) x-ray detector with a resolution of about 145 eV for the 5.9-keV manganese K-x rays was used to detect the fluorescent x rays from the target. The detector was placed at 135° to the incident beam with the entrance window 50.0 mm from the beam spot. A beam energy of 2.5 MeV and a beam current of 2-3 nA were used throughout the course of the experiment. Measurement times were ~ 40 min for the measurements on the brain sections.

The MDLs for elements heavier than aluminum which could be achieved under these conditions were measured using a target of National Bureau of Standards Standard Reference Material, No. 1571, orchard leaves. The value for aluminum was found with the standards prepared in the manner described above.

**RESULTS**

The values found for the MDLs as a function of atomic number are shown in Fig. 2. The MDL is defined in terms of the Currie criterion (12) as $3.29 \cdot C\sqrt{Nbg}/N$, where N and Nbg are the number of counts in an elemental x-ray peak and in the background under the peak, respectively, and C is the elemental concentration. The MDL found for aluminum using an aluminum standard prepared as described above was 16 ppm μg/g. It corresponds to detection of $3 \times 10^9$ atoms in the $20 \times 20 \times 20$ $\mu m^3$ volume of tissue which was probed.

The microPIXE spectra obtained from the cerebral cortex in an aluminum-intoxicated rat and a control rat are shown in Figs. 3 and 4. The aluminum x-ray peak is clearly present in the first and absent in the second. The spectra also show the multielemental nature of the PIXE method with sever

different elements clearly visible. Correlations of aluminum content with the concentrations of other elements are easily made.

The data obtained are given in Table 1. The $^{14}$C-2DG uptake was significantly lower in the cerebral cortical regions which corresponded to areas of increased aluminum content. This decreased glucose uptake was also seen in the hippocampus of Al-treated rats.

## DISCUSSION

The combined results obtained using the autoradiography measurement of $^{14}$C to estimate the uptake of $^{14}$C-2DG and microPIXE to obtain the elemental concentrations show that there is an inverse correlation between the aluminum concentration in the brain and decreased brain glucose metabolism. The value of the mean ratio of $^{14}$C-2DG activity to aluminum concentration changes by a factor of 8 or more between the experimental and control brain sections. This change is a factor of four more than is found for fluctuations in the individual points measured. From examination of the spectra shown in Figs. 2 and 3, it can be seen that there are also suggestive changes in the concentrations of the other elements observed. There is a suggestion of an inverse correlation of potassium and calcium with aluminum, but no dependence is seen for magnesium.

The induction of aluminum encephalopathy (AE) in the rat permits investigation of the temporal changes in behavioral and electroencephalographic (EEG) indices along with measurements of brain elemental content and glucose uptake in aluminum-treated and control rats. Behavioral and EEG changes have been measured in these rats repeatedly at different times following graded doses of aluminum. Results of those studies

are presented in a separate report (4). Measurements of regional elemental content and autoradiographic (ARG) assessment of glucose uptake require sacrificing of the animal, and hence only a single temporal estimation can be obtained from each animal. Results of experimental animal studies are useful in providing guidance with respect to relevant measurements to be made in human subjects, along with the number of observations needed based on accuracy and reproducibility of the observations. Studies reported herein have shown the feasibility of making measurements using currently available BNL microPIXE facilities to determine the content of Al, Mg, P, Si, S, Cl, K, Ca, Fe, and Cu in thin sections (< 30 μm) of biological tissues. Further, it is possible in these sections to make other nondestructive analyses on the same sample such as the quantitative ARG images of glucose uptake performed in these studies or radioreceptor quantitation as analyzed by others (13).

The results of these studies are consistent with observations in humans studied with Positron Emission Tomography (PETT), which reveal diminished cerebral cortical glucose metabolism in patients with Alzheimer's Disease (14). In addition, the observation that aluminum content is increased in the neurofibrillary tangles (NFTs) in Alzheimer's Disease, along with calcium has led to the suggestion that these increases could be explained by assuming the presence of hyperparathyroidism secondary to dietary deprivation of calcium and magnesium in selected populations (2). Silicon accumulation has also been reported in NFTs, but this could not be tested in our studies due to contamination of samples with talc used in the ARG preparations.

The feasibility of making highly sensitive accurate measurements of aluminum and other elements in human material with microPIXE has been demonstrated. The problem in the analysis of Al content in patients with

Alzheimer's disease is that the increased Al content appears to be localized mainly in the small NFTs which are ~ 1 $\mu m^3$ in size. Thus a decreased spot size for the exciting beam and thinner samples could be useful in maximizing measurement sensitivity. The determination of the MDLs showed that it was possible to obtain measurements on aluminum using a 20-μm beam diameter, in cases where the concentration is greater than 16 ppm g/g.

The sensitivity of the PIXE system appears to be adequate for the high levels of Al found in the massively dosed rats. However, the minimum detectable limit of 16 ppm is too high for use in human studies where levels in the 5-15 ppm range are of greatest interest. Furthermore, the fact that Perl (5) has shown that Al in Alzheimer's disease is localized in the intracellular extranuclear neurofibrillary tangles (NFT), makes the present 20-μm beam size unsuitable for studies in that disease. The more diffuse cerebral cortical localization in the dialysis dementia patients, and in the rat model we studied, however, can be studied with PIXE.

The major technological problem that remains is how to choose the regions in which to make the large number of measurements that would be needed as one realizes it is not possible to survey an entire brain. If one attempted to study an entire rat brain (~ 1 $cm^3$), the number of 10 μm-diameter spots per 10-μm thick section is $10^6$, and there would be $10^3$ such sections to be analyzed. If each assay required only 1 sec, a complete survey would take approximately $10^9$ sec or approximately 25 years to analyze. Clearly that is not possible for even small samples, and hence sampling guidance criteria are needed. These are provided frequently by the occurrence of pathological changes, as the NFTs in Alzheimer's disease. In other cases, where functional changes are suspect, without detectable pathological changes, the situation is

more complex. In that case, animal models such as we have used could be employed using autoradiography employing radiolabeled indicators or fluorescent dye labeled tracers. In regions where functional abnormalities are noted, the trace element or microanalytic assays could be employed and results contrasted to anatomical structures in which no functional changes were noted. Such techniques require the administration in life of the material to a subject whose post mortem studies are closely related in time. The practical and ethical aspects of such studies preclude their use in human subjects. However, it is possible to do single and double tracer studies with radiolabeled and fluorescent labeled compounds on post mortem tissues from patients. Such a method was used by Altar, et al. (13) to image dopamine and serotonin receptors in the brain using $^{3}$H-spiroperidol in the presence and absence of specific receptor blocking agents. The localization of such receptors could guide the trace element investigator to test the correlation of trace element alterations with changes in receptor site density or activity. Other competitive binding systems could be employed to choose sampling strategies aimed at characterizing interactions between toxins and biological functioning of systems at lower levels than are pathologically detectable.

## SUMMARY

The applicability of microPIXE methods to trace element studies dealing with aluminum and other trace element measurements in tissue sections has been demonstrated. The techniques require scrupulous care in sample preparation to avoid contamination. The ability to choose the size and location of regions of interest to be probed are of paramount importance, in view of the need to

relate measurements to biological functions, and anatomical regions, as well as the practical limitations imposed by the time available for measurement and data analysis.

The studies conducted on the rat model of aluminum encephalopathy have indicated some of the problems and potential advantages associated with these methods. The preliminary results which indicate a correlation between Al level in the brain and decreased brain glucose metabolism are in accord with observations in patients with Alzheimer's disease (14). Further studies directed at the level of the lesion (1-μm resolution) with $^{3}$H-2DG could be useful if a proper animal model of Alzheimer's disease were available.

**ACKNOWLEDGMENTS**

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Table 1. Summary of concentration measurements of $^{14}C$ 2 deoxy-D-glucose uptake and aluminum concentrations observed in rat brain.

| | $^{14}C$ 2 Deoxy-D-Glucose Uptake (nCi/g) | | | $^{27}Al$ Concentration ($g/g \times 10^{-6}$) | | |
|---|---|---|---|---|---|---|
| | Al-T | Na-T | $\frac{\text{Na-T}}{\text{Al-T}}$ | Al-T | Na-T | $\frac{\text{Na-T}}{\text{Al-T}}$ |
| Cerebral Cortex | 82 | 134 | 1.64 | 66 | <16 | >8.4 |
| Range | (54-105) | (116-150) | ---- | 38-83 | | |
| Hippocampus | 97 | 144 | 1.48 | ---- | ---- | ---- |

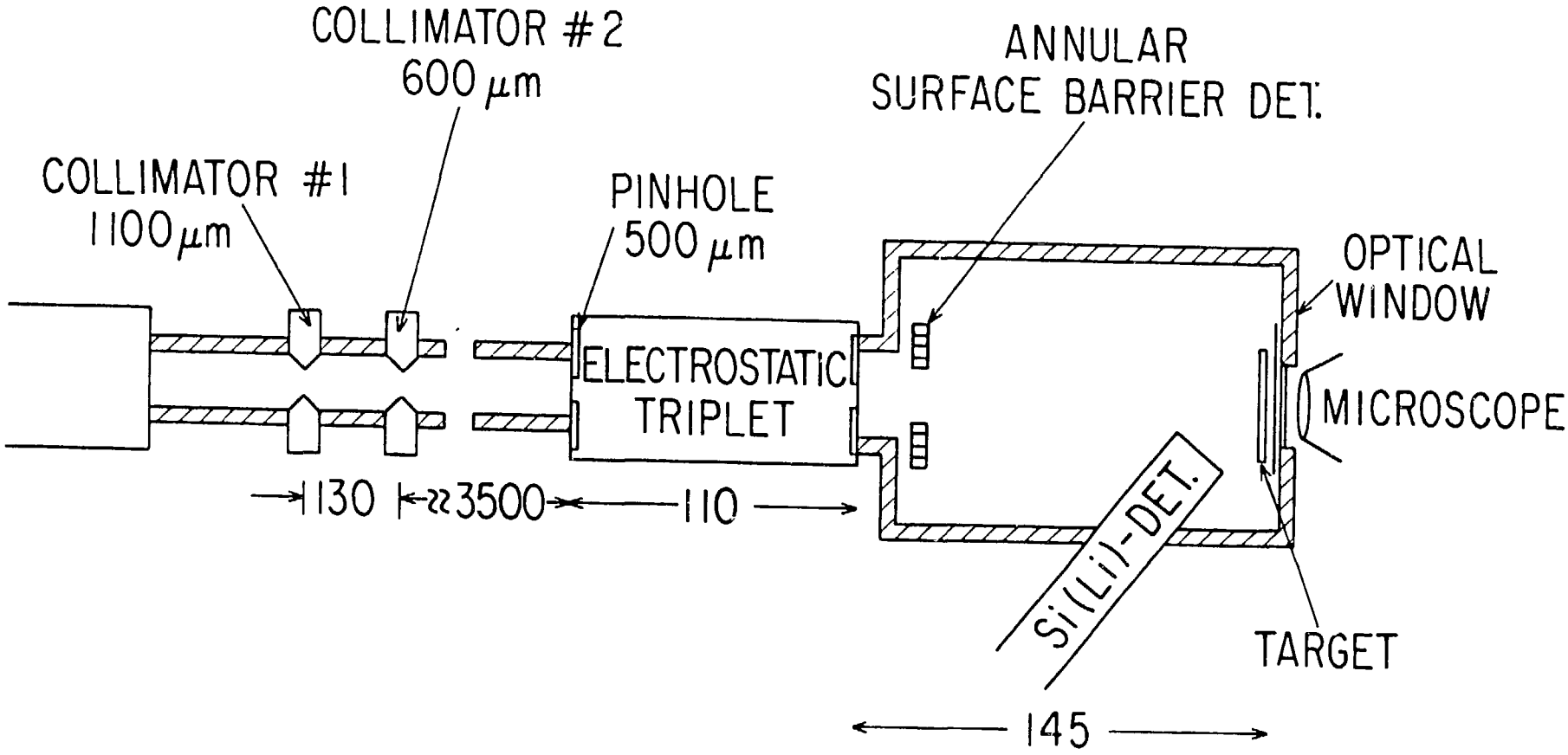
**FIGURE CAPTIONS**

Figure 1. Schematic diagram of the experimental apparatus used in the microPIXE determinations.

Figure 2. Minimum detectable limits for the microPIXE beam used in this experiment.

Figure 3. X-ray spectrum obtained from control rat. The high content of Si is an artifact due to the use of Si-containing powder in the ARG process.

Figure 4. Typical spectrum obtained from Al-treated rats. The observed concentration of Al varied from 38 to 83 ppm. The aluminum peak is shown in the expanded part of the spectrum.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Si(Li)-DET.
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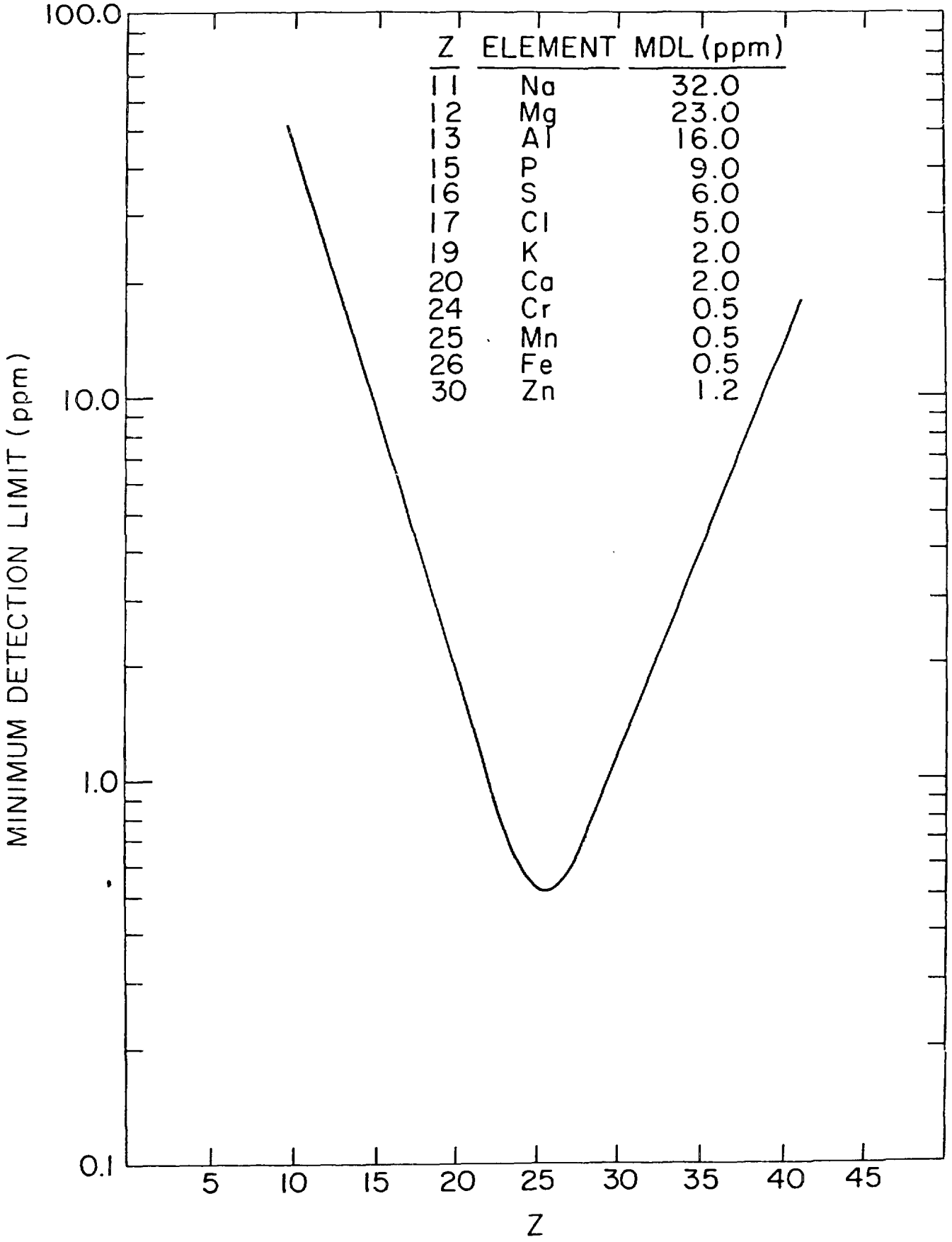
Figure 1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Z ELEMENT MDL (ppm)
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12 Mg 23.0
13 Al 16.0
15 P 9.0
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25 Mn 0.5
26 Fe 0.5
30 Zn 1.2

Figure 2

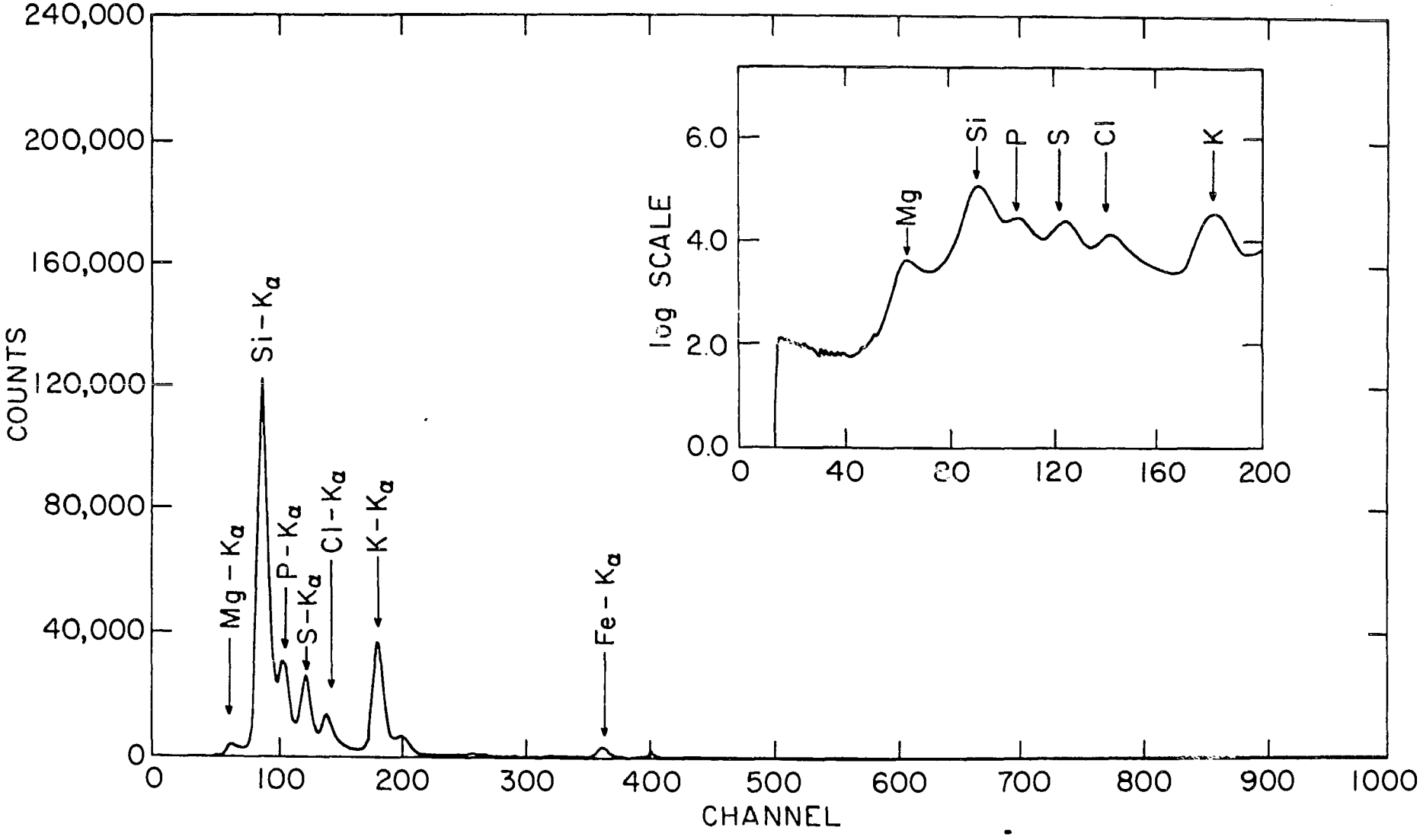
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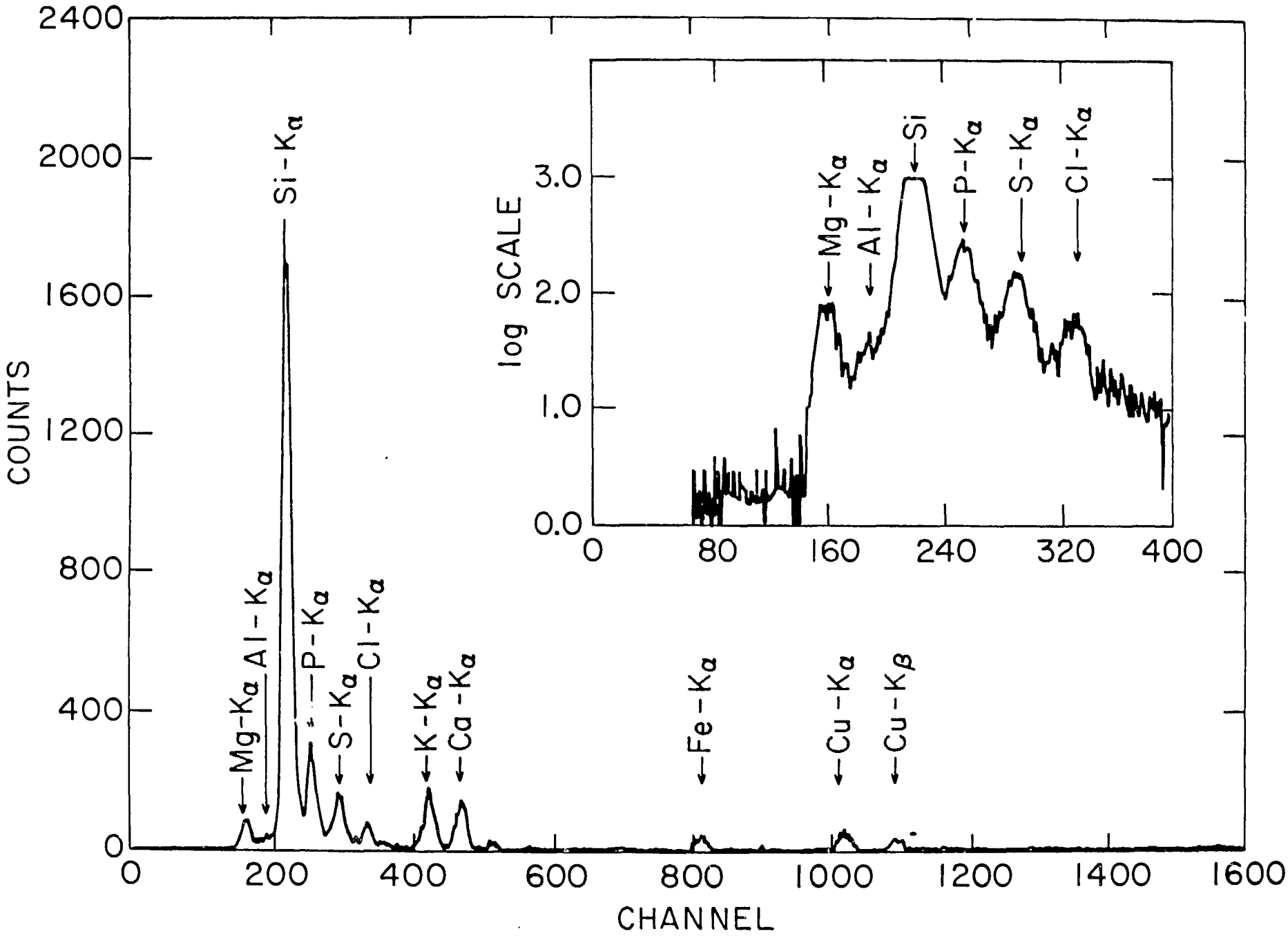
Figure 3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Cu-Kβ
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