0163-4984/92/3403–0249 $02.00

# Proton-Induced X-Ray Emission Analysis of Atherosclerotic Plaques of the Carotid Bifurcation

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Received August 22, 1991; Accepted September 30, 1991

## ABSTRACT

The trace elements of both calcified atherosclerotic plaques and plaque-free vessel walls of the carotid bifurcation from 31 autopsies were investigated using the proton-induced X-ray emission (PIXE) method. The trace elements studied were phosphorus (P), calcium (Ca), chrome (Cr), iron (Fe), copper (Cu), zinc (Zn), lead (Pb), selenium (Se), bromine (Br), strontium (Sr), and rubidium (Rb). All samples contained Fe and Zn. Mercury (Hg) was not detected in any of the samples studied. All plaque-free samples contained Cu and almost all Br and Ca, none Sr. All calcified atherosclerotic plaques contained Ca and almost all Br and Sr. The relative levels of Ca were higher in the calcified plaques than in the plaque-free vessel walls. The relative value of Ca in calcified and uncalcified samples was greatest in the group who had died because of cardiovascular disorders and smallest in the group who had died from other causes. There was a strong positive correlation between the Ca and Sr of the plaque samples and between the P and Br of the plaque-free samples.

**Index Entries:** Atherosclerosis; PIXE.

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## INTRODUCTION

Atherosclerosis is a local pathological process in the walls of arteries. An atheroma arises when cholesterol begins to accumulate in the arterial intima, forming a cholesterol nidus. In the late stage of atherosclerosis, calcium salts also accumulate into an atheromatous plaque (*1*). Development of atherosclerosis of the common carotid artery begins as early as that in the aorta and coronary arteries. Advanced changes in the carotid bifurcation may be seen in persons only 20 yr old (*2*).

We have earlier investigated the prevalence and extent of atherosclerotic calcifications of the carotid bifurcation in a Finnish autopsy material using a low-kV radiographic method to visualize calcifications and a special computerized method to calculate the extent of these changes (*3*). However, no further analyses of the calcifications were possible with these methods.

Proton-induced X-ray emission analysis is a reliable way of determining the presence of trace elements in biological samples (*4–7*). Its advantages include low detection limits, multielemental capacity, and the small size of the sample required (*5,8,9*). Proton-induced X-ray emission has ben used to investigate atherosclerotic changes in the aorta and arteria basilaris (*4,10*).

In this study, we used PIXE to study atherosclerotic changes in carotid bifurcation and the plaque-free vessel wall. Special attention was given to Ca, Fe, Zn, Br, Sr, and Cu levels; but P, Pb, Se, Rb, and Cr were also determined. As far as we know, no other investigation has determined such a wide range of elements in calcification of the carotid bifurcation.

## MATERIALS AND METHODS

The material consisted of carotid bifurcations obtained from randomly selected autopsies at the Institute of Forensic Medicine, University of Helsinki. The specimens were taken from 31 persons (12 women and 19 men). The mean age was 70 yr (range: 52–93 yr). Two samples were taken from every vessel: one from the calcified plaque and the other from plaque-free intima. The samples were dried in a gel slab drier (Pharmacia GSD-4) between two cellophane films and analyzed from the intimal side. For the plaque analyses, the intima covering the calcified plaque was removed to get direct contact between the proton beam and the calcification.

The proton beam was generated in a 2.4 MeV van de Graaff accelerator at the University of Helsinki. The accelerator was equipped with a 90° analysing magnet having an ion-path radius of 60 cm. The protons were yexcited through a thin Kapton® foil (2.5 μm) using a method described earlier (*11,12*). The X-rays were detected with a 50 $mm^2$ × 6 mm intrinsic

Ge-detector with an energy resolution of 170 eV. The collected charge ($Q$) was 45 μC, current 150 mA, and the diameter of the proton beam 3.5 mm. In the measurements, each sample was bombarded for 5 min.

The PIXE method is based on bombardment of the samples with 2.4 MeV protons, which displace inner shell electrons from target atoms. The vacancies are filled by less tightly bound electrons, which can be observed as emission of characteristic X-rays. Thus, the elements of the samples can be identified from the positions of the peaks in the spectrum and their levels determined from the peak areas. The spectra were stored in an Osborne M 286 V computer. The areas of the peaks representing the relative levels of the different trace elements were calculated. The spectra were plotted using the GEN PLOT program (Cornell University, Department of Material Science and Engineering, Ithaca, NY),

Every effort was made to avoid contamination of the samples. They were prepared using titanium (T) instruments because the T peak is clearly detectable in the spectrum, and there is no problem in its interpretation. Tests showed that the cellophane film used contributed no additional peaks to the spectrum. The samples were divided into groups according to the subject's age, sex, cause of death, and profession. In 22 cases, the cause of death was cardiovascular disease (including 14 cases of myocardial infarction), in four cases alcohol, and in five cases some other reason. The professions were divided into two categories: manual and office work.

## RESULTS

Figure 1 shows a typical spectrum obtained from a plaque-free vessel wall and Fig. 2 a typical spectrum for a calcified plaque. Table 1 shows the prevalence of the trace elements and the range of their relative levels. All samples contained Fe and Zn. All plaque-free samples contained Cu, 30 Br, and 29 Ca. Three of the plaque-free samples contained Cr, but none of them contained Sr. All calcified plaques contained Ca and 30 of them contained Br and Sr. Twenty of the calcified plaques contained Cu. In a few cases of both plaque-free and plaque samples, there were also smaller amounts of P, Pb, Se, and Rb. Other substances, for instance Hg, were not present or were below the detection limit. In one case, there was an extra peak in the spectrum caused by gold (Au). Further studies revealed that the person in question was a rheumatic treated with gold.

The statistically significant correlations between the trace elements are presented in Table 2. There was a strong positive correlation between Ca and Sr in the plaque samples and between P and Br in the plaque-free samples. The mean relative levels of Ca in both calcified and plaque-free samples was greatest in the group who had died because of cardiovascular disorders and smallest in the group who died from other causes. No other correlations were seen between the cause of death and trace ele-

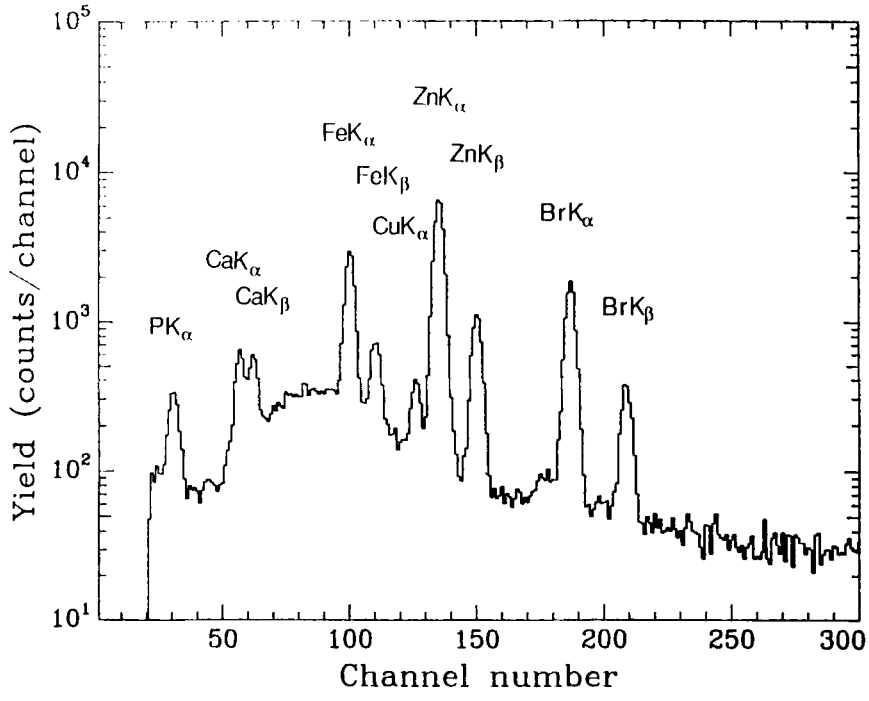


Fig. 1. A typical spectrum for a plaque-free vessel wall.

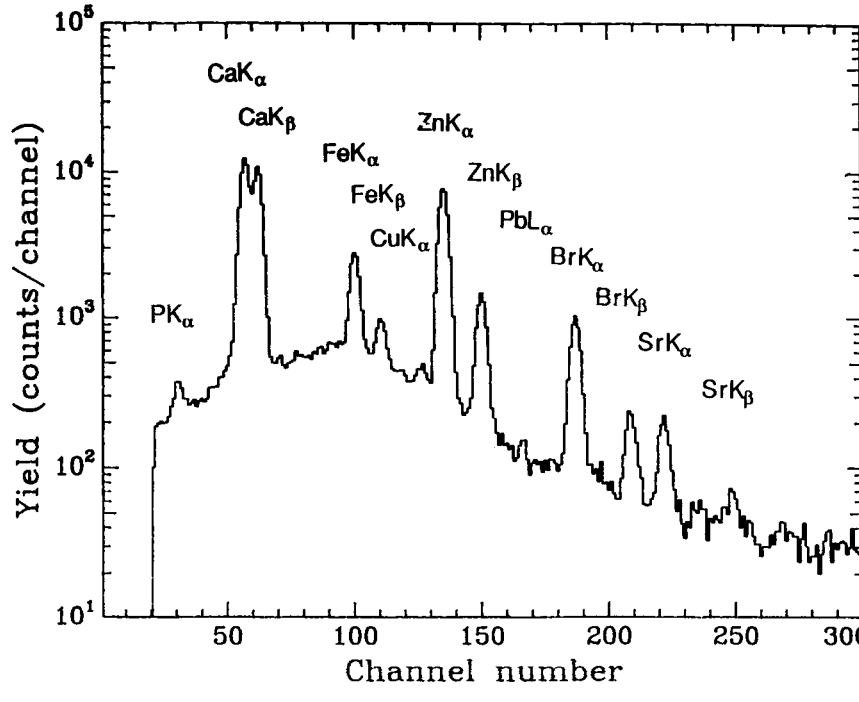


Fig. 2. A typical spectrum for a calcified atherosclerotic plaque.

Table 1
Prevalence of Relative Levels of Trace Elements
in 31 Calcified Atherosclerotic Plaques and Plaque-Free Vessel Wall

| Plaque-free vessel wall | | | | Calcified atherosclerotic plaque | | | |
|---|---|---|---|---|---|---|---|
| | | Range of values | | | | Range of values | |
| Trace element | *n* | Min | Max | Trace element | *n* | Min | Max |
| P | 21 | 313 | 1330 | P | 13 | 292 | 2300 |
| Ca | 29 | 730 | 7700 | Ca | 31 | 7960 | 386,000 |
| Cr | 3 | 615 | 1650 | Cr | — | — | — |
| Fe | 31 | 10,700 | 194,000 | Fe | 31 | 5080 | 178,000 |
| Cu | 31 | 656 | 2060 | Cu | 20 | 182 | 1810 |
| Zn | 31 | 897 | 5230 | Zn | 31 | 1240 | 20,800 |
| Pb | 4 | 133 | 210 | Pb | 10 | 175 | 1660 |
| Se | 18 | 92 | 278 | Se | 5 | 64 | 185 |
| Br | 30 | 248 | 8450 | Br | 30 | 465 | 5280 |
| Sr | — | — | — | Sr | 30 | 121 | 2990 |
| Rb | 6 | 119 | 215 | Rb | 6 | 63 | 484 |

*n* = number of cases.

Table 2
Statistically Significant Correlations Between Trace Elements
in Plaque-Free and Plaque Samples

| Plaque-free vessel wall | | | Calcified atherosclerotic plaque | | |
|---|---|---|---|---|---|
| Element | *n* | *r* | Element | *n* | *r* |
| P/Ca** | 21 | 0.517 | P/Br** | 13 | 0.650 |
| P/Zn* | 21 | 0.416 | Ca/Cu* | 20 | −0.442 |
| P/Br*** | 21 | 0.942 | Ca/Zn*** | 31 | 0.851 |
| P/Rb* | 6 | 0.737 | Ca/Br*** | 30 | −0.553 |
| Ca/Fe* | 29 | −0.331 | Ca/Sr*** | 30 | 0.913 |
| Ca/Zn** | 29 | 0.433 | Ca/Rb* | 6 | 0.762 |
| Ca/Br*** | 29 | 0.570 | Fe/Rb* | 6 | 0.859 |
| Fe/Rb** | 6 | 0.885 | Cu/Br*** | 20 | 0.710 |
| Zn/Se* | 18 | 0.441 | Cu/Sr* | 19 | −0.464 |
| Zn/Br*** | 30 | 0.663 | Zn/Br* | 30 | −0.397 |
| Zn/Rb* | 6 | 0.849 | Zn/Sr*** | 30 | 0.764 |
| Br/Rb* | 6 | 0.733 | Zn/Rb** | 6 | 0.912 |
| | | | Pb/Sr* | 10 | 0.597 |
| | | | Br/Sr** | 29 | −0.490 |

* = $p < 0.05$;
** = $p < 0.01$;
*** = $p < 0.001$;
*n* = number of cases; and
*r* = correlation coefficient.

ment compositions in this study. Because of the small number of subjects, classification according to age, sex, and profession was not possible, and no statistically significant differences were detected.

## DISCUSSION

With the plaque samples, the International Atomic Energy Agency (IAEA) bone standard was used to obtain the absolute concentrations of different trace elements. This could not be done for the plaque-free samples because of the lack of a suitable standard. For this reason, only the relative levels of the trace elements were used in the plaque-free and plaque samples.

In carotid bifurcations, Sr was present in almost all atherosclerotic areas but never in the plaque-free vessel wall. The relative values of Ca were higher in the plaque that in the healthy arterial wall. This is in agreement with the findings of Guffey et al. (*4*), who noticed that the damaged tissue contained higher amounts of Ca and Sr than the healthy tissue. In the study of Badica et al. (*10*), concentrations of Ca and Cu were significantly higher in arteries with atheromatous lesions than in healthy arterial walls. In our study, the difference of Cu values between plaque and plaque-free samples was not significant.

The reason why three of the plaque-free samples contained Cr was not clear. There were two plaque-free samples that did not contain any Ca. In one of the cases, the reason might be high age (93 yr). In the other cases, the reason is unknown. The reason why one plaque-free sample and one plaque sample contained no Br is also unexplained.

This investigation, like Guffey's and Badica's, involved only a small number of samples. Our samples were randomly selected just to test the method and to shed light upon the different trace elements present in plaques. However, we found a slight difference in the relative levels of Ca in the healthy vessel wall and in the calcified atherosclerotic plaques between those who had died because of cardiovascular disorders and alcoholics. These results justify continuing the examination with a larger material in special groups, for example, persons who have died of myocardial infarction and those who have died of alcoholism. A comparison between the plaques of healthy persons and those who have died because of malignancies could be of interest, too.

## ACKNOWLEDGMENTS

The authors express their warm thanks to Matti Hyttinen for his help in preparing the cadavers and to Raimo Ingren for the smooth running of the accelerator.

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