X-RAY SPECTROSCOPY IN BIOLOGY AND MEDICINE. VI USE OF AN ULTRA-SOFT X-RAY SOURCE FOR THE NONDESTRUCTIVE DETECTION OF MICROGRAM AMOUNTS OF SODIUM AND MAGNESIUM

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The continuous short wavelength X-rays produced in the primary beam from X-ray tubes constructed with the usual tungsten and chromium targets are inefficient in exciting the K_{α} lines of low atomic number elements (numbers 6 through 12). This has prevented the micro-analysis of these important elements of biological origin, using commercially available X-ray spectrographic equipment. Recently, Henke (1) has developed an X-ray tube fitted with an aluminum target and a thin aluminum foil window that is an efficient source of ultra-soft X-rays. Using this X-ray source, which can be readily incorporated into available instruments, the authors have been able to demonstrate the feasibility of the nondestructive measurement of sodium and magnesium at the microgram level in biological material.

Methods

Specimens were prepared for presentation to the spectrograph as dried films on aluminum foil planchets or in filter paper disks as described previously (2-6). A

Norelco vacuum path X-ray spectrograph with associated electronic circuit panel and pulse height analyzer was used. The aluminum target X-ray tube was fitted with a 6 micron aluminum window, and was operated at 10 KV and 150 MA by means of an auxiliary power supply (1). A gypsum analyzing crystal was used with no exit port collimation and the pressure in the X-ray path was held at less than 200 microns of mercury. Detector and gain settings were adjusted to permit pulse-height analysis, using a baseline of 7.5 volts with window voltages of 15.6 and 18 for sodium and magnesium, respectively. Line intensities were quantitated with a gas-flow proportional detector with P-10 as the filling gas. The detector was fitted with a 6 micron aluminum window.

Results

Human liver and blood serum were chosen as representative biological specimens for this initial study. Specimens were scanned qualitatively from 9 to 12 A and in every instance the K_{α} lines of sodium and magnesium were observed with signal/background counting rates that conformed with statistical requirements for valid quantitation in a reasonable period of time. An example of such a scan is presented in Figure 1 for a specimen of liver. This presentation consisted of a dried film representing 5 mg. of tissue wet weight and contained about 3-5 μg. of sodium and magnesium.

Appropriate background and line settings were selected, using these qualitative scans. Background was estimated

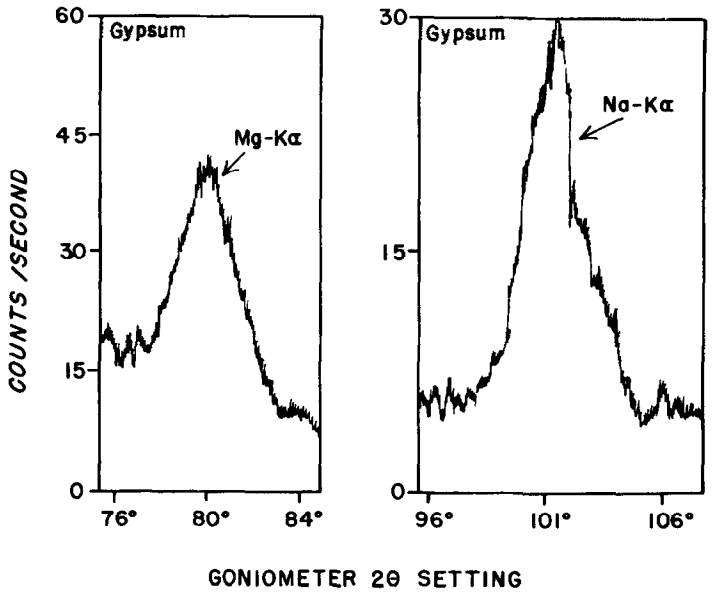

Figure 1. Sodium and magnesium in 5 mg. of human liver. The specimen was hydrolyzed briefly in nitric acid and dried as a thin film on an aluminum foil planchet for X-ray evaluation.

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at Bragg angle settings near the lines to be measured. Since the background for magnesium is sloping, counts were taken in the region immediately above and below the angular setting of this line, and the values were averaged. Counts were collected at 101.7° for the K_α line of sodium and at 79.8° for the K_α line of magnesium. Results obtained are presented in Table 1. Peak to background ratios for 50 microliters of serum were 15 and 2.8 to 1 for sodium and magnesium, respectively. In terms of total amount of element, this volume of serum contains about 150 micrograms of sodium and approximately 2 micrograms of magnesium. Results obtained with 5 mg. of liver were similarly encouraging, despite

TABLE 1

COUNTING RATES OF THE SODIUM AND MAGNESIUM IN SERUM AND LIVER*

	Sodium			Magnesium		
Specimen	Bkgd.	Line	Net	Bkgd.	Line	Net
Serum, 50 μl. on foil	8.4	124.8	116.4	15.2	42.7	27.5
Blank foil	8.4	8.9	0.5	20.3	17.2	-3.1
Liver, 5 mg. on foil	8.6	31.0	22.4	13.7	41.5	27.8
Reagent blank on foil	9.3	17.7	8.4	17.1	31.0	13.9
Liver, 25 mg. on paper	5.8	11.5	5.7	6.2	22.8	16.6

*Values are given in counts/second. The sodium background was estimated at 106.0° while the magnesium background was the average counting rate at 75.5° and 84.0°. The specimens on foil were distributed as a dry film over a 13 mm. circular area. The specimen on paper was dried in a 13 mm. disk of Whatman No. 40 filter paper.

the fact that the amount of tissue selected was suboptimal, and that submicrogram, but significant, reagent contamination was indicated.

Discussion

It is evident that a considerable gain in sensitivity of detection has been obtained for the low atomic number elements, sodium and magnesium, by using an excitation

source yielding a high flux of X-rays with wavelengths in the region of their critical absorption edges. Although these results are highly encouraging, certain refinements will be necessary before this type of analysis can be applied in a quantitative sense. A degree of caution is indicated since, with the specimens employed, a high proportion of the measured characteristic X-ray quanta probably originates in the most superficial layers of the specimen. Losses of the desired X-ray quanta originating at greater depths within the specimen will be increasingly significant. This is clearly demonstrated by the low signal yield for 25 mg. of liver mounted in filter paper (Table 1). This effect becomes more important, the longer the wavelength of the radiation being measured.

Conclusion

Using an aluminum target X-ray tube as a high-yield source of ultra-soft primary X-rays, the nondestructive X-ray spectrographic detection of microgram amounts of sodium and magnesium in biological materials has been demonstrated.

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