Micro-Determination of Bromine in Biological Samples Using Proton-Induced X-Ray Emission Spectroscopy

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HONDA, K., SHISHIDO, S., SUZUKI, T., KUBO, H. and CHIBA, R. *Micro-Determination of Bromine in Biological Samples Using Proton-Induced X-Ray Emission Spectroscopy*. Tohoku J. exp. Med., 1975, 117 (1), 89-91 — The plasma level of bromine was measured by a proton-induced X-ray emission spectroscopy. Since bromine is the most difficult halogen to be determined and appears only in trace quantities, powerful techniques are required. The excellent reproducibility was obtained on 19 measurements on a sample of a single individual; the coefficient of variation was 6.3%. The average and standard deviation of plasma bromine levels on 47 young women were 0.50 and 0.10 mg/100 ml, respectively. Results by other methods in the literature were briefly reviewed. ——— bromine; plasma bromine level

Elements of the halogen group in nature are absorbed together by plants and animals, and should be present as normal constituents of the living tissues. The presence, level, and function of chlorine, iodine and fluorine in the human body have been well known, while the presence of bromine has been denied for a long time, and even after recognition of the presence, its level and function in the human body have been a matter of controversy. The reason why bromine has been rather neglected in its significance for physiological meanings is the difficulty of determining its quantity which is trace.

Some investigators used a colorimetric method; e.g. the auric chloride method (Gray et al. 1942), which is useful for large quantities of bromine in blood or urine, as frequently encountered in the case of bromide poisoning (Natelson and Clark 1955). In the biological specimens from cases without any particular intake of bromine, substances that react with auric chloride like bromine, give a brown color, and interfere with the determination of bromine, become a problem. The bromate-roosaniline method (Hunter 1955) improved the specificity of determination of

bromine, but it brought about a tedious incineration and a possible loss of bromine during the incineration.

The neutron activation analysis is recommended because it is sensitive and less subject to interference by other halogens (Ohno 1971; Bowen 1959). But, the problem is an accessibility to a nuclear reactor. The proton-induced X-ray emission spectroscopy requires only a small amount of sample and provides an adequate sensitivity and specificity for determination of bromine (Valkovic et al. 1974; Jundt 1974; Kubo et al. 1974; Chiba 1975), and the apparatus to supply a proton beam is rather easily accessible as compared with the nuclear reactor. Then, the application of this technique to biological samples is tested in this study.

EXPERIMENTAL METHODS

Apparatus. A 2.25-MeV proton beam from a Van de Graff accelerator (Tokyo Institute of Technology) with an intensity of $0.1 \mu\text{a}$.

Target preparation. A thin film of Formvar set on an aluminum frame (16 cm^2 , 1 mm thick) with a central hole of 3.1 cm diameter was used as a target backing. Ten to $20 \mu\text{l}$ of plasma or other biological sample solution and an equal volume of $400 \mu\text{g Sr/ml}$ solution as standard were deposited on this Formvar film and evaporated to dry.

Detector of X-ray. A Si (Li) semiconductor detector.

Determination of bromine concentration. On X-ray spectra obtained by proton bombardment of targets, the bromine concentration was calculated from a proportion of its peak area to a standard peak area in consideration of difference between element sensitivities against proton bombardment.

RESULTS

Reproducibility of determination. On 19 measurements on a sample of a single individual, the coefficient of variation was 6.3% ($0.48 \pm 0.03 \text{ mg/100 ml}$ of plasma).

Levels of bromine in human plasma. The average and standard deviations of plasma bromine levels on 47 young women aged 18 or 19, were 0.50 and 0.10 mg/100 ml, respectively.

Application to blood of mouse or rat (a preliminary result). Plasma bromine levels on a rat and a mouse were 5.7, and 4.8 mg/100 ml, respectively (blood was obtained by heart puncture).

DISCUSSION

Some of the advantages of this technique over other methods of bromine analysis are that (a) it is rapid, (b) it has an excellent reproducibility, (c) it requires only a small amount of sample, (d) it is more sensitive and more specific, and (e) no chemical preparation or ashing is necessary.

Bowen (1959) reported that blood bromine level was $0.28 \pm 0.01 \text{ mg/100 ml}$ ($n=7$) by neutron activation analysis. Since erythrocytes contain few bromine, plasma bromine level is calculated as about $0.56 \pm 0.02 \text{ mg/100 ml}$. Goodwin (1971) measured serum bromine levels by a bromate-rosaniline method without incine-

ration; a modified method of Hunter's (1955). Bromine values obtained ranged from 0 to 3.05 mg/100 ml (average, 0.75; standard deviation, 0.90 mg/100 ml). Our present results are in agreement with those by the neutron activation analysis (Bowen 1959), not those by the bromate-rosaniline method (Goodwin 1971).

Moreover, there were methods by iodometric titration (Conway and Flood 1936) or colorimetry using fluorescein (Ucko 1936) which were applied to human blood. The values by those methods were higher in the iodometric titration, or lower in the fluorescein colorimetry than those by recent physical methods.

The determination on plasma of rats or mice was only preliminary and the observed levels of bromine were about 10 times higher than that in human plasma. The results by Hunter's bromate-rosaniline method on rat's plasma (Logten et al. 1973, 1974) were 1.76 ± 0.88 mg/100 ml ($n=4$). For experimental animals like rats or mice, further elucidation is necessary.

References

- 1) Bowen, H.J.M. (1959) The determination of chlorine, bromine and iodine in biological material by activation analysis. *Biochem. J.*, **73**, 381-384.
 - 2) Chiba, R. (1975) Application of element analyses using an accelerator to medicine. *Igaku no Ayumi* (Jap.), **92**, 297-300.
 - 3) Conway, E.J. & Flood, J.C. (1936) The micro-determination of bromine, with application to blood and urine and observations on the normal human subject. *Biochem. J.*, **30**, 716-727.
 - 4) Goodwin, J.F. (1971) Colorimetric measurement of serum bromide with a bromate-rosaniline method. *Clin. Chem.*, **17**, 544-547.
 - 5) Gray, M.G., Moore, M. & Mass, B. (1942) Blood bromine determinations: Their use and interpretation. *J. Lab. clin. Med.*, **7**, 680-686.
 - 6) Hunter, G. (1955) Micro-determination of bromide in body fluids. *Biochem. J.*, **60**, 261-264.
 - 7) Jundt, F.C. (1974) Proton-induced X-ray analysis of trace elements in tissue sections. *J. Histochem. Cytochem.*, **22**, 1-6.
 - 8) Kubo, H., Hashimoto, S., Chiba, R. & Nakamura, H. (1974) Use of a Si (Li) semiconductor detector for trace element analysis in human tissue samples. *Radioisotopes* (Jap.), **23**, 42-46.
 - 9) Logten, M.J.Van, Wolthuis, M., Rauws, A.G. & Kroes, R. (1973) Short-term toxicity study on sodium bromide in rats. *Toxicology*, **1**, 321-327.
 - 10) Logten, M.J.Van, Wolthuis, M., Rauws, A.G., Kroes, R., Tonkelaar, E.M.D., Berkvens, H. & Esch, G.J.Van (1974) Semichronic toxicity study of sodium bromide in rats. *Toxicology*, **2**, 257-267.
 - 11) Natelson, S. & Clark, K. (1955) Estimation of bromide on fingertip blood in bromide intoxication. *Proc. Soc. exp. Biol. Med. (N.Y.)*, **90**, 723-725.
 - 12) Ohno, S. (1971) Determination of iodine and bromine in biological materials by neutron-activation analysis. *Analyst*, **96**, 432-436.
 - 13) Ucko, H. (1936) Investigations into the presence and the role of bromine in the body. *Biochem. J.*, **30**, 992-1000.
 - 14) Valkovic, V., Liebert, R.B., Zabel, T., Larson, H.T., Miljanic, D., Wheeler, R.M. & Phillips, G.C. (1974) Trace element analysis using proton-induced X-ray emission spectroscopy. *Nucl. Instr. & Meth.*, **114**, 573-579.
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