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Blood Bromine Levels in a Pacific Atoll Population¹

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Serum and red cells from 20 Marshallese on two atolls and from 10 subjects in New York were compared for their elemental composition employing an energy dispersive X-ray fluorescence technique. The elements analyzed in serum included Cl, Zn, and Br, whereas red cells were analyzed for Cl, K, Fe, Zn, Br, and Rb. Both Marshallese groups showed statistically significant ($P < 0.01$) elevations in serum Br (51 and 96%) and in red cell Br (393 and 478%) as compared to the New York group. The Marshallese Br/Cl ratio in serum and in red cells was elevated when compared to that of the New York group, whereas the Rb/K ratios were equivalent. The red cell Br/serum Br ratio was also elevated in the Marshallese subjects. There were no similar differences noted among the other elements tested. Drinking liquids in the Marshall Islands were analyzed for Br but did not provide a clear source for the elevated Br levels. © 1986 Academic Press, Inc.

INTRODUCTION

Blood Br levels measured in several populations have been found to be similar (1-6). Although Br is not considered to be an essential trace element (7), its normal distribution in tissue is similar to the distribution of essential trace elements (8, 9).

A recent study of the elemental composition of serum and red cells in a group of 20 individuals from the mid-Pacific Republic of the Marshall Islands and 10 New Yorkers revealed that Br was singularly elevated in the Marshallese group. The investigation used the technique of energy dispersive X-ray fluorescence (EDXRF). This report presents details of the elemental measurements of serum and red cells together with water samples taken from different locations.

SUBJECTS AND METHODS

Subjects. Thirty adults undergoing routine medical examinations were evaluated. Ten individuals were employees of Brookhaven National Laboratory, Long Island, New York. Twenty individuals were inhabitants of the Republic of the Marshall Islands. Of the latter, 10 lived on the remote atoll of Rongelap and 10 lived on Majuro atoll, location of the capital of the Republic and a well-populated district center. All groups were approximately age-matched and had a similar number of males and females. The Marshallese subjects receive periodic medical examinations and health care through a long-established program carried out by the Medical Department of Brookhaven National Laboratory. The purpose of the program is to diagnose and treat radiation-related illness resulting from accidental

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exposure of some Marshallese to radioactive fallout in 1954 (10). Nevertheless, the program encompasses many aspects of health care not related to radiation.

Specimen collection. Nonfasting blood samples were collected at the time of medical examination in New York, Rongelap, or Majuro. The venipuncture site was cleansed with 70% isopropyl alcohol (Tomac Prep Packet, Amer. Hosp. Supply, Edison, N.J.). Multisample Vacutainer blood collection tubes Model B-D 6453, containing disodium EDTA, and Model B-D 6512 (Becton, Dickinson and Co., Rutherford, N.J.) were used for red cells and serum, respectively. Packed red cells were obtained after washing the anticoagulated blood three times with normal saline (0.9% sodium chloride injection, Travenol, Deerfield, Ill.). Red cells and sera were frozen in polyethylene chemical containers (Bel-Art, Pequannour, N.J.) for transport and storage. The same type of vial was used for storage of atoll water and coconut liquid collected for the evaluation of environmental sources of Br in drinking fluids.

Specimen analysis. In preparation for analysis 300- μ l samples of thawed serum or red cells were layered onto Parafilm over an area of 1 cm². The specimens were then covered to avoid dust contamination and air-dried. Sample processing was alternated among the groups of specimens to minimize systematic bias in preparation or analysis.

For EDXRF analysis the sample was irradiated with soft X rays emitted by a ¹⁰⁹Cd radioactive source. This technique creates vacancies in the inner atomic shells of the elements. The subsequent emission of characteristic X rays by the target atoms is monitored by a Si(Li) detector. The signals are processed by standard electronics and displayed in the form of a pulse-height distribution. This method enables quantitative and qualitative evaluation of the sample composition. In the present study, red cells were analyzed for Cl, K, Fe, Zn, Br, and Rb, and the sera were analyzed for Cl, Zn, and Br. Other elements were below the detection limits of the EDXRF system employed.

Calibration. The EDXRF system was calibrated for Br with the addition method in which serum and red cell samples were spiked with graded amounts of Br (5 to 15 μ g/ml) in the form of NaBrO₃. It was determined that the mean value of 3296 counts of Br corresponds to a concentration of 1.3 μ g Br/ml of packed red cells (the calibration line is $\text{ppm} = 4.02 \times 10^{-4} \text{XRF} - 4.83 \times 10^{-4}$ where XRF is the Br peak intensity) and a mean value of 23,971 counts corresponded to 6.4 μ g Br/ml of serum (calibration line is $\text{ppm} = 2.62 \cdot 10^{-4} \text{XRF} + 8.33 \cdot 10^{-2}$). These values were used to calculate absolute Br concentrations in the blood samples.

Statistical analysis. For analysis of normalized values of all the detected elements, a standard one way analysis of variance (ANOVA) was used. The *F* test was applied to test the equality of group means, and *t* statistics were computed to test the equality of means between each pair of groups. The nonparametric Wilcoxon rank sum test and Spearman's rank correlation were used for analysis of group differences in serum and red cell Br levels.

RESULTS

Possible contamination of containers was investigated. Vacutainers are known to be contaminated with inconsequential amounts of Br (<0.006 and <0.05 μ g per

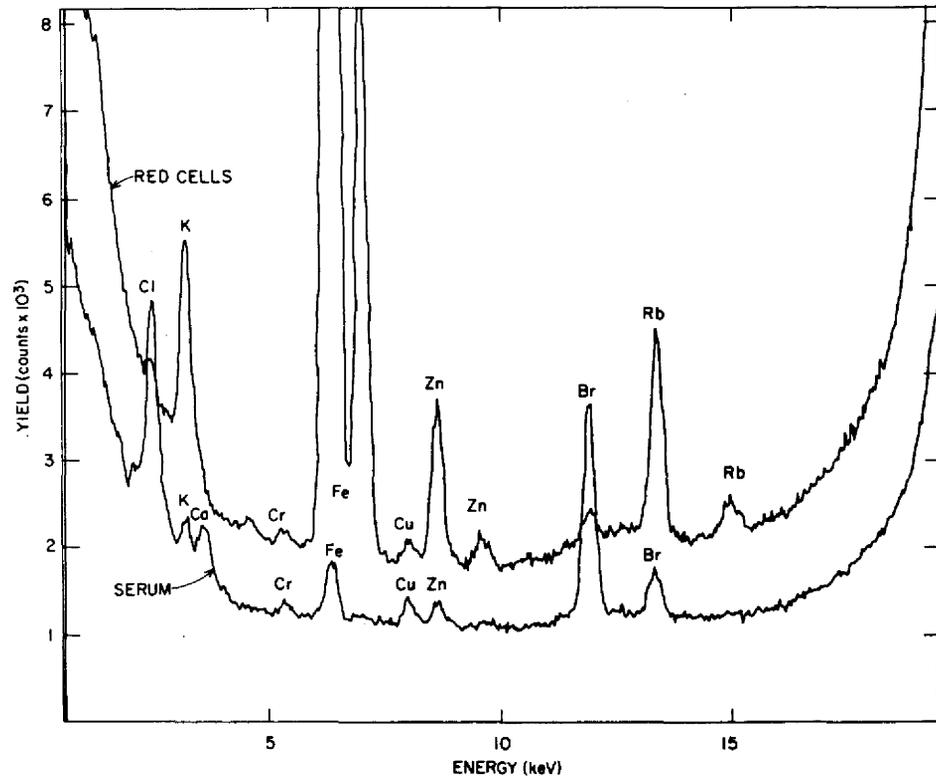


FIG. 1. Typical EDXRF spectrum of red cells and serum from a New York subject.

nonanticoagulated and EDTA-anticoagulated tube, respectively (11)). It was further determined that the serum separator of Vacutainer tube Model B-D 6512 contains no Br. Water samples which were transported in polyethylene containers similar to those used for serum and red cell frozen storage had no detectable Br.

Additional evidence that contamination did not account for the elevated Br levels in the blood samples come from data on three New York subjects whose serum and red cell specimens were prepared in the Republic of the Marshall Islands concurrently with the Marshallese specimens. All three serum Br values of the New York subjects were lower than the lowest Marshallese serum level; moreover, the mean value of the three red cell Br concentrations was lower than that of the mean Marshallese value. It was concluded, therefore, that Br contamination was not a factor in the analysis.

Typical EDXRF spectra from red cells and serum samples, taken from a New York subject, are shown in Fig. 1. The peak intensities (peak area) are proportional to the corresponding elemental concentration in the sample. The results of the analyses for each element normalized to the mean value of the New York group are shown in Fig. 2. The relative group means for each element tested are summarized in Table 1. The striking elevation of serum and red cell Br in the Marshallese was unique among the different elements evaluated by EDXRF.

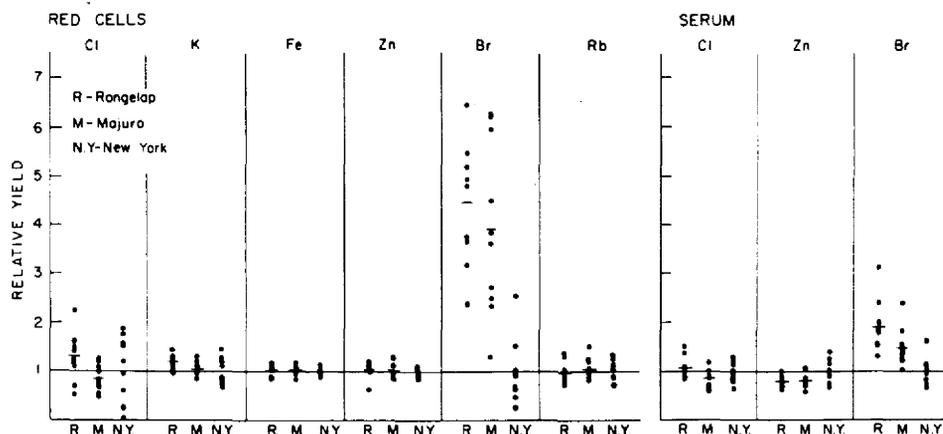


FIG. 2. Relative abundance of the elements present in the red cells and the serum normalized by the mean value of the New York group.

The mean serum Br levels in the Rongelap and Majuro groups were 96 and 51% higher, respectively, than that of the New York group. Mean red cell Br concentrations of the Marshallese were also elevated over that of the New Yorkers: 478% in the Rongelap group and 393% in the Majuro group. On the basis of the Wilcoxon rank sum test, the Rongelap serum Br level was significantly higher than that of the Majuro group ($P < 0.02$) which, in turn, was significantly higher than that of the New Yorkers ($P < 0.01$). The red cell Br levels of the two Marshallese groups were not significantly different from each other, but were significantly higher than that of the New Yorkers ($P < 0.01$).

TABLE 1
NORMALIZED GROUP MEAN VALUES FOR ELEMENTS ANALYZED BY ENERGY DISPERSIVE
X-RAY FLUORESCENCE (EDXRF)

Group element	Rongelap	Majuro	New York	Normalization factor*
Red cells				
Cl	1.32	0.86	1.00	2,222
K	1.22	1.06	1.00	23,379
Fe	1.03	1.03	1.00	463,955
Zn	1.07	1.04	1.00	16,903
Br	4.78	3.93	1.00	3,296
Rb	0.97	1.05	1.00	18,121
Serum				
Cl	1.06	0.84	1.00	16,249
Zn	0.80	0.83	1.00	2,108
Br	1.96	1.51	1.00	23,971

* The EDXRF mean peak intensity in the New York group.

The Br concentration in Rongelap lagoon water was similar to that of Atlantic ocean water (62.4 ppm and 67.2 ppm, respectively). Rain catchment water on Rongelap had no detectable Br. Intermediate Br values of 2.2, 13.8, and 17.9 ppm were found in three Rongelap wells. The Br concentration in three samples of Rongelap coconut water was 7.0, 6.4, and 5.3 ppm, similar to the 7.0 and 6.8 ppm found in two Florida-grown coconuts.

DISCUSSION

The mean serum and red cell Br levels of the 20 Marshallese studied were significantly higher than those of the 10 New Yorkers, as well as being higher than levels reported for other populations (1-6). Cross and Smith (8) have suggested that the tissue ratio of Br to Cl is determined physiologically and that elevated ratios indicate excessive Br intake. The mean Br/Cl ratios of 1.83 for serum and 4.1 for red cells in the Marshallese were higher than the New York ratios (which were arbitrarily set at unity). This finding suggests excessive Br intake by the Marshallese. It is of interest to point out that Rb, which is considered to be a K analog just as Br is considered to be Cl analog, had similar ratio Rb/K in all three populations studied.

Tissue Br can be elevated by an increase in the dietary Br intake and also by respiratory and cutaneous exposures (4). Leaded gasoline, which contains organic bromide, is little used on Rongelap, where Br levels were highest, and can be ruled out as a significant Br source. There is no atmospheric pollution with Br-containing dust or industrial fumes. Some residual fumigant Br may be present on imported U.S. Department of Agriculture foods on Rongelap, but these foods are not supplied on Majuro. Therefore, a local environmental source of Br was suspected.

Ocean water has a bromine concentration of 65.0 ppm (12), consistent with the measurements of Atlantic ocean and lagoon water sampled for the present study. There is significant vertical exchange of lagoon and ocean water with the fresh water lens system of atoll islands (13), and dug wells exhibit different degrees of salinity depending on their depth and placement about the lens. The variable levels of Br detected in well water are consistent with earlier atoll studies of Cl and other elements (unpublished 1972 water quality data from Bikini and Enewetak atolls kindly supplied by C. J. Huxel, U.S. Dept. of the Interior Geological Survey, Honolulu, Hawaii). However, the most important source of drinking water is rain catchment water, samples of which showed no Br present. Another commonly ingested liquid is coconut water, which is used for cooking as well as for drinking. An estimate of the weight of coconut products ingested daily, including coconut water, is 2.4 kg for a man aged 23-50 years of age (14). The Br concentrations measured in coconut water samples would provide an average daily consumption of 17 mg Br solely from coconuts. The acceptable daily intake of Br set by the FAO/WHO is 1 mg/kg body weight (15). It has been suggested that a more appropriate value would be 0.1 mg/kg body wt (16), which would approximate one-third of a Marshallese man's daily intake of Br from coconuts alone. Br levels in other food supplied were not tested in the present study, but

TABLE 2
BLOOD Br LEVELS

	Men	Women	Mean age (years)	(A) Mean serum Br ± SD (mg/liter)	(B) Mean red cell Br ± SD (mg/liter)	(B)/(A) ± SD	Spearman correlation coefficient (<i>r</i>), serum vs red cell Br
Rongelap	5	5	44.9	12.54 ± 3.16	6.12 ± 1.21	0.49 ± 0.16	+0.09(NS)*
Majuro	4	6	44.1	9.63 ± 2.31	5.03 ± 2.13	0.52 ± 0.25	+0.29(NS)
New York	6	4	42.5	6.39 ± 1.67	1.28 ± 0.79	0.20 ± 0.13	-0.09(NS)

* NS = Net significance.

one source of dietary bromine, seaweed, is not normally a part of the Marshallese diet.

Essential and nonessential trace elements are often distinguishable by normal and log-normal concentration distributions, respectively (9). A normal distribution for bromine was found for both the New Yorkers and the Marshallese, in that the means of each of the three groups' values were similar to their respective median values. Similar findings have been reported from Scotland (8). However, prolonged exposure to absorbed trace elements can result in elevated but stable tissue levels and a normal distribution (9).

A strong correlation between serum and red cell Br levels ($P < 0.01$) was noted, a finding also reported by Stump *et al.* (5). This correlation was observed for the combined data from all 30 subjects, but was not found within any of the three subgroups (see Table 2). More than simple diffusion may be involved in bromine partition *in vivo* because the red cell to serum Br ratio of the Marshallese was more than twice that of the New Yorkers (Table 2). These data are not predicted by *in vivo* Br tracer studies. Gamble *et al.* (17) noted that, following the intravenous injection of $\text{NH}_4^{82}\text{Br}$ in man, equilibrium between plasma and red cell Br was reached within one hour. The red cell to serum Br ratio they reported was 0.54, a value similar to that found in the Marshallese (Table 2). The low ratio found in the New Yorkers may therefore indicate a nonlinear relation between serum and red cell Br at lower levels.

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