

DETERMINATION OF EXTRACELLULAR FLUID VOLUME BY FLUORESCENT EXCITATION ANALYSIS OF BROMINE

Leon Kaufman and Carol Jane Wilson

University of California, San Francisco, California

The measurement of extracellular fluid volume (ECFV) is important in the study of body composition in patients and research animals. We present a simplified method using fluorescent excitation analysis for measuring nonradioactive bromine in plasma which provides a means for estimation of ECFV. This method is fast and accurate and does not require the administration of radioactive substances.

We have found the measurement of extracellular fluid volume (ECFV) to be useful in the evaluation of the cardiovascular and nutritional status of children with uremia (1). Others have evaluated ECFV in normally growing children, children with growth retardation, infants of low birth weight, and malnourished children (2-5). Changes in body composition in adults with severe uremia have been measured and used to assess rehabilitation with hemodialysis (6). Previous methods of measuring ECFV using dilution techniques with ^{36}Cl , ^{38}Cl , ^{82}Br , and stable bromine have had the disadvantage either of exposing the patient to radiation or of requiring difficult chemical analysis. This paper describes an easily performed method for determining the extracellular fluid volume as represented by the corrected bromide space using characteristic fluorescent excitation analysis (7). Bromine concentration in small volumes of plasma are measured rapidly and accurately without radiation exposure to the subject.

METHODS AND MATERIALS

Fluorescent excitation. The technique for measuring bromine is an adaptation of a method developed for iodine (8). A 2.5-mCi cyclotron-grade ^{109}Cd source of 0.3 cm diam is placed in a holder/collimator which limits photon emission to a well-defined beam. Cadmium-109 is chosen because its emission energies of 21.10 and 24.99 keV allow for the ex-

citation of bromine without overlap of Compton-scattered photons with the 11.91-keV K_α x-ray of bromine. Figure 1 illustrates the typical excitation spectrum obtained from a solution containing bromine. The K_α and K_β lines of bromine lie in a region of minimum background between the Compton-scattered photons (C) and the characteristic lines of the materials of the collimators, holders, etc. (L). An 80-mm² Si(Li) KeVex detector with a 3-mm depletion depth is collimated to limit its acceptance to a narrow beam. The source and detector collimators are positioned perpendicular to one another, producing a 1-cm³ region of sensitivity within which the sample to be analyzed is placed (Fig. 2). Pulses from the detector are fed into a Tennelec 2002 BLR amplifier and stored in a 1,024 multichannel Packard 900 Analyzer (MCA). As described here, the system is on wheels and is fully portable.

For determination of bromine concentration, the number of counts in the K_α peak are totaled by the MCA and are compared with the total in a narrow region of the Compton peak (Fig. 1). The bromine concentration of a sample is given by

$$\text{Concentration} = K \left(\frac{NK_\alpha}{NC} - B \right). \quad (1)$$

In this formula, the counts in the K_α and Compton channels are expressed by NK_α and NC , respectively, and B represents the NK_α/NC ratio for a water blank. The factor K is a calibration constant obtained by the measurement of a bromine sample of known concentration. Because the system is remarkably stable, the constants K and B need to be measured only once for a particular sample container and detector configuration. Concentration may be ex-

Received Dec. 21, 1972; revision accepted June 26, 1973.

For reprints contact: Leon Kaufman, Nuclear Medicine Section, Dept. of Radiology, University of California, San Francisco, Calif. 94143.

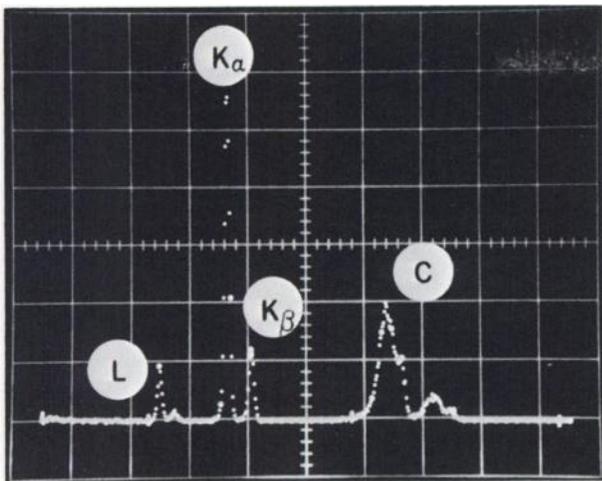


FIG. 1. Typical ^{109}Cd excitation spectrum of bromine solution obtained at 90-deg scattering angle. Bromine K_{α} and K_{β} peaks lie in region of minimum background between Compton peak (C) and characteristic lines of materials of collimators, holders, etc. (L).

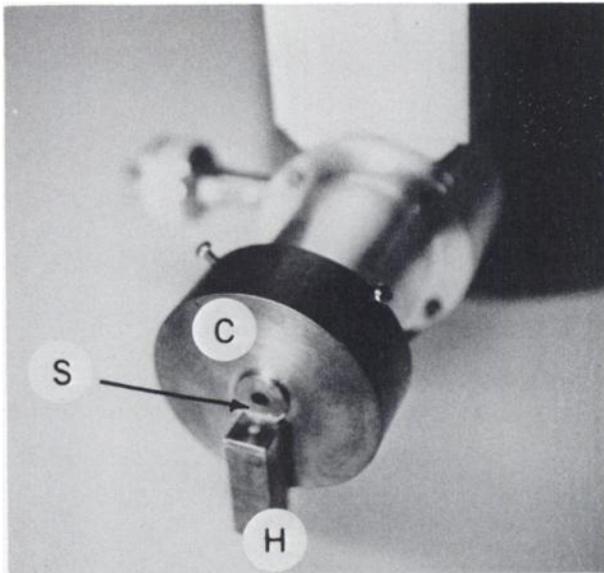


FIG. 2. Si(Li) detector with collimator (C), and holder/collimator for ^{109}Cd source (H). Sample is positioned within sensitive region defined by two collimators (S).

pressed in any units which are convenient (e.g., mEq/liter, mg/cc). The results obtained by this method are independent of sample positioning, detector efficiency, sample volume, and instrumentation deadtime. Counting time affects only the statistical accuracy of the final result. Because of these factors, this method provides an extremely reliable way to obtain bromine determinations over a wide range of concentrations. The process can be automated with the addition of a sample changer and a simple arithmetic circuit to yield concentrations directly in the desired units (9).

Extracellular fluid volume. The ECFV of humans

and rats was estimated by the corrected bromide space in an adaptation of the method described by Cheek (10). A weighed volume of sterile 3% sodium bromide was administered intravenously. The amount to be injected was calculated to produce a plasma bromide concentration of approximately 1–2 mEq/liter. After equilibration, blood was obtained for bromide determination; routinely, samples were obtained after 3 and 4 hr in humans and after 3 hr in rats. Bromide concentration in plasma water was obtained by dividing plasma bromide by the plasma water content determined by desiccation to constant weight and dividing by the Donnan factor (0.95). In the normal individual, 6% of the injected bromide is equilibrated within erythrocytes and another 4% is distributed outside the extracellular space, within cells, cerebral spinal fluid, and the GI tract; this non-extracellular fraction must be subtracted from the total injected bromide in calculating the extracellular bromide space (Eq. 2). In anemic individuals, the amount of bromide in erythrocytes has been determined in our laboratory to be proportional to the hematocrit; therefore, correction for erythrocyte bromide can be made on the basis of hematocrit.

Extracellular bromide (mEq) =

$$\text{mEq injected} - \left[4\% \text{ mEq injected} + 6\% \left(\frac{\text{Observed hematocrit}}{40} \right) \right]. \quad (2)$$

The corrected bromide space is then calculated by

Corrected bromide space (L) =

$$\frac{\text{Extracellular bromide (mEq)}}{\text{Bromide in plasma water (mEq/liter)}}. \quad (3)$$

For comparison with the fluorescent excitation method, the corrected bromide space in a series of patients was also calculated from bromide concentrations obtained chemically using Conway's microdiffusion technique (11).

RESULTS

To determine the response of the system, a set of standards containing accurately known concentrations of bromine was made up. Concentrations of 0.025–500 mEq/liter resulted in a linear response with a random variation of $\pm 2\%$. The accuracy of the method is dependent upon the accumulation of counts in the K_{α} channel, and the statistical precision of an analysis is determined by the counting time and bromine concentration. Table 1 shows the results of 14 replicate determinations of standards containing 1.75 and 292 mEq/liter using two different counting times. As expected, reproducibility was

TABLE 1. ACCURACY OF CONCENTRATION DETERMINATION

Method	Counting time (sec)	Concentration (mEq/liter)	Standard deviation (%)
Chemical	*	1.42	2.6
	20	292	1.3
	50	292	0.8
Fluorescent	200	1.75	4.7
	500	1.75	2.6

* Typically eight samples are analyzed in an 8-hr day.

TABLE 2. COMPARISON OF FLUORESCENT AND CHEMICAL DETERMINATIONS

	Corrected bromide space (liters)		
	Fluorescent	Chemical	Dif. %
Patients			
1	3.45	3.62	4.7
2	13.12	11.63	-11.3
3	5.70	5.60	-1.7
4	9.45	9.08	-3.9
5	6.28	5.90	-6.0
6	6.59	7.28	10.5
Rats			
1	0.121	0.127	5.4
2	0.136	0.141	3.5
Mean			6.7

improved with increased counting times, and a percent standard deviation (coefficient of variation) of less than 3% was achieved in a reasonable counting period. The reproducibility of the chemical method is illustrated for comparison; a coefficient of variation of 2.6% was obtained in a series of 94 determinations. The use of a 20-mCi ^{100}Cd source has improved system performance so that a sample with a bromine concentration of 2 mEq/liter can be measured with an accuracy of 1.6% in a time of 100 sec (as compared with 2.6% in .500 sec as shown in Table 1).

The corrected bromide spaces determined by the two methods in six infant patients and two rats are compared with Table 2. The expected variation between the two methods would be about 5%; in these determinations, we obtained a standard deviation of 6.7%. There was not a statistically significant difference demonstrable between the two methods using the paired t-test. In five cases, the fluorescent excitation determination was repeated after samples had been frozen in plastic containers for 3-14 days. The change in the calculated bromide space averaged 6% and varied from 0 to $\pm 10\%$.

DISCUSSION

Bromine measurements may now be made rapidly and accurately with the development of fluorescent

excitation analysis. To perform the chemical micro-diffusion determination of stable bromine for duplicate samples from two patient studies requires a full day's work by a careful, well-trained technician. In contrast, after a brief training session, an unskilled operator can determine bromine levels in a like number of samples in less than an hour with fluorescent excitation. Such rapid readout of results will increase the use of the ECFV measurement and its application in diverse clinical situations.

The volume of plasma required for bromine determinations with this technique is small. We have been using 0.25-1.0 ml, but with modification of the container, as little as 0.1 ml can be used. Thus this method lends itself to the study of newborn and premature infants with fluid volume problems. In rats in which bromide toxicity may not be of concern, a higher dose of sodium bromide can be injected and plasma diluted for determination. Because only stable bromide is used, the patient is not exposed to radiation and the injected bromide and the plasma samples do not require special handling. Since administered bromine levels are of the order of 5-10% of those where symptomatic bromism appears and normal clearance is of the order of 3%/day, serial studies may be performed in one individual without concern for either cumulative radiation exposure or toxicity. In patients with renal failure, bromine is cleared rapidly by dialysis. The presence of usual diagnostic amounts of an isotope or of any other substance does not interfere with the analysis of bromine. Fluorescent excitation analysis does not change the plasma; therefore, more than one study may be carried out upon a single sample. We are presently using this method in estimating the corrected bromide space in children. The versatility of fluorescent excitation analysis is demonstrated by the possibility of its use in determining the bromide content of tissues other than plasma. We are presently using the method for determination of the extracellular fluid space of various organs.

ACKNOWLEDGMENTS

We wish to thank Malcolm R. Powell, Head, Nuclear Medicine Section, for first suggesting the applicability of the fluorescent method to ECFV determination and Malcolm Holliday, Professor of Pediatrics, for his encouragement of this work. We gratefully acknowledge the meticulous work of Macario Buzon who performed the chemical analyses. We also wish to thank Richard Frankel and Rolf Woldseth of the KeVex Corporation for the loan of the Si(Li) detector used in this work. We have been supported in part by funds from USPHS GM 01272 of the National Institute of General Medical Sciences, and by Training Grant HD-00182 from the National Institute of Child Health and Human Development, and by a USPHS Research Career Development Award (Number GM 70598-01) from the NIGMS.

REFERENCES

1. WILSON CJ, POTTER DE, HOLLIDAY MA: An approach to the comprehensive treatment of children with chronic renal failure. In *The Body Fluids in Pediatrics*, Winters RW, ed, Boston, Little Brown, 1972
2. CHEEK DB: Extracellular volume: Its structure and measurement and the influence of age and disease. *J Pediatr* 58: 103-125, 1961
3. FRIIS HANSEN B: Body water compartments in children: Changes during growth and related changes in body composition. *Pediatrics* 28: 169-181, 1961
4. CASSADY G: Bromide space studies in infants of low birth weight. *Pediatr Res* 4: 14-24, 1970
5. GRAHAM GG, CORDANO A, BLIZZARD RM, et al: Infantile malnutrition: Changes in body composition during rehabilitation. *Pediatr Res* 3: 579-589, 1969
6. COLES GA: Body composition in chronic renal failure. *Q J Med* 41: 25-47, 1972
7. BECK RN, GOTTSCHALK A, HOFFER PB: *Semiconductor Detector in the Future of Nuclear Medicine*, New York, Society of Nuclear Medicine, 1970
8. MOSS A, KAUFMAN L, NELSON JA: Fluorescent excitation analysis: A method of iodine determinations in vitro. *Invest Radiol* 7: 335-338, 1972
9. WILLIAMS G: An automated system is presently under development. Private communication, Northern Scientific, Madison, Wis
10. CHEEK DB: *Human Growth*, Philadelphia, Lea and Febiger, 1968
11. CHEEK DB: Estimation of the bromide space with a modification of Conway's method. *J Appl Physiol* 5: 639-644, 1953

**TECHNOLOGIST SECTION
THE SOCIETY OF NUCLEAR MEDICINE
21st ANNUAL MEETING**

June 11-14, 1974

Town and Country Hotel

San Diego, California

2nd CALL FOR SCIENTIFIC EXHIBITS:

NUCLEAR MEDICINE TECHNOLOGISTS' PROGRAM

The Technologist Scientific Sessions Committee announces that abstracts of exhibits are now being reviewed for the 21st Annual Meeting. Abstracts of exhibits are welcomed from technical affiliates.

All exhibits will be illuminated by available room light. There will be no provisions for transillumination, e.g., view boxes. The exhibit should be mounted on poster board not exceeding 30 in. X 30 in. No more than two boards may be entered for a subject. Exhibits should be clearly titled.

Abstract format: Exhibitor's name; title of exhibit (10 words maximum); abstract (100 words); dimensions (A maximum of two boards not exceeding 30 in. X 30 in.).

Exhibit Awards: The section is pleased to announce the presentation of 1st, 2nd and 3rd place awards for the three most outstanding scientific exhibits. These are judged on the basis of scientific merit, originality, display format, and appearance.

MARK I. MUILENBURG
Nuclear Medicine
Creighton Memorial St. Joseph's Hospital
Omaha, Nebraska 68134

DEADLINE: April 15, 1974